

Results of Crossing *Euschistus variolarius* and *Euschistus servus* with reference to the Inheritance of an Exclusively Male Character. By KATHARINE FOOT and E. C. STROBELL. (Communicated by Prof. E. B. POULTON, F.R.S., Pres.L.S.)

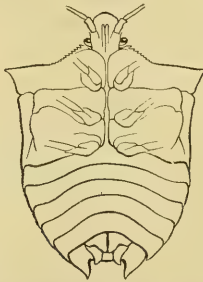
(PLATES 28-34, and 2 Text-figures.)

[Read 5th March, 1914.]

EUSCHISTUS VARIOLARIUS has an exclusively male character which is not present in *Euschistus servus*, and it was the hope of being able to study the transmission of this character, and its bearing on modern chromosome theories, that led us to attempt to cross these two species*.

This specific character is a distinct black spot on the male genital segment of *E. variolarius*, and as there is no spot on the genital segment of the female (text-fig. 1), this spot in *E. variolarius* is an exclusively male character.

Fig. 1.



Euschistus variolarius ♀. A comparison of this sketch with the male *variolarius* of photo 1 (Pl. 28), will show the marked difference in the form of the genital segment of the two sexes, and will demonstrate that the genital spot characteristic of the male could not appear in the female without a modification of the entire genital segment.

The two species (*variolarius* and *servus*) are shown in photo 1 (Pl. 28). On the left is a male *variolarius*, and on the right a male *servus*. The spot on the male genital segment of *variolarius* is clearly demonstrated, and the complete absence of such a spot on the male genital segment of *servus* is clearly shown. This spot in *variolarius* is a constant character: it appears as distinctly in all the males of this species as in the seven specimens shown in photo 2. Five male specimens of *E. servus* are shown in photo 3 (Pl. 30).

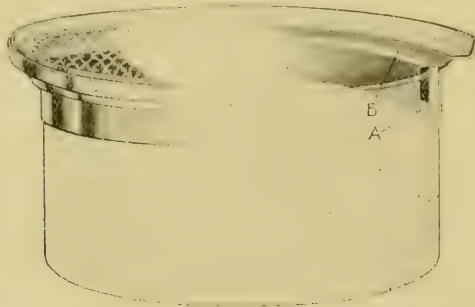
The difficulties involved in raising Hemiptera in captivity are serious. For five consecutive summers we have experimented raising several species

* The *E. variolarius* ♀ used for the cross-breeding experiments was raised in our laboratory from material we collected at Ridgefield, Connecticut, and the *E. servus* were collected at Southern Pines, North Carolina, by Rev. A. H. Manee.

in our laboratory, aiming to learn to raise these bugs with as low a death-rate as possible, and it was not until after three years of experimenting that we felt it would be safe to attempt to cross two or more of these species. The three most troublesome problems in this work were to provide the proper food, to use cages well adapted for cleanliness and observation, and to maintain the right degree of moisture. The bugs must have not only abundant food, but it must be fresh and clean, and these conditions can be successfully met only by frequently transferring the insects to clean cages with fresh food. This should be done at least every third day, or, better still, every second day. We were forced to do this work ourselves, for raising the nymphs demands such delicate care that we were unable to entrust it to an assistant. It proved to be an arduous task during part of the breeding season, when, in our experiments, we found it necessary to change the food for more than 30 cages each day.

The cages we used for the adult bugs were glass dishes about 6 inches in diameter and 3 inches deep. The covers were of coarse brass wire mesh, carefully designed to prevent the possibility of the tarsi of the bugs being caught at any point where the brass cover comes in contact with the glass dish (text-fig. 2). Until we were able to design these covers the bugs were

Fig. 2.



Cage designed to prevent the insects from catching the tarsi at the point of contact between the glass dish and the metal top. The spaces marked A and B are large enough to allow the legs to move about freely, but are too small to admit any part of the body.

frequently mutilated, for if the tarsus is caught, the entire leg of the bug is sacrificed, for the leg always gives way at the proximal end of the femur.

Such mutilation apparently does not seriously interfere with the functional activity of the bugs, for in one case a female that had lost three legs continued to mate and lay eggs quite normally. The greatest danger lies in the fact that a bug in this mutilated condition cannot always regain its normal position if it has, by chance, dropped on its back, for in this position even the adult bugs frequently die in a few hours.

The food chosen for our experiments was wheat and orchard grass while

they lasted, and later in the season timothy heads and berries. The former were placed in the cage in two small bunches, each containing not more than five heads, cut to about 3 inches in length and the stems tightly wrapped together in wet absorbent cotton. If the cotton is kept wet, the food will keep quite fresh for three days.

When blackberries were used, they were not plucked from the stems, but small sprays with berries were selected, cut in lengths of 3 inches, and the stems wrapped in wet cotton. All the leaves were carefully trimmed from each spray, and only one large, or two small sprays were used in each cage. The leaves must be cut off because they hide the bugs, and they are of no value as food—becoming dry in a few hours. It is very important to place the food in the cages in such a way that every bug can be seen at any time and closely watched. When we used strawberries or cherries, we suspended them by their stems from the wire tops of the cage. Frequently we used the tips of young milk-weed, for we found that the bugs in captivity often deposit their eggs on the under side of these leaves, though we have never found any deposited on milk-weed in nature. In order to be sure that all the eggs of our experiments were deposited by the bugs we had under observation, no fresh food was ever placed in the cages without being carefully examined to be sure that no eggs of a kindred species were by any chance brought into the cages. If, however, the food is changed every third day, this danger is practically eliminated, for the eggs of this species require only from 5 to 7 days to hatch, and unless any alien eggs were deposited on the food the same day it was put in the cage, such a mistake would be very readily detected. This particular risk to our experiments was reduced to a minimum in the season of 1912, for the locality where we spent the summer was not only an unfavourable one for Hemiptera, but the exceptional cold of the preceding winter had made the locality even more than usually unfavourable. During the entire summer we succeeded in finding only two specimens of *Euschistus variolarius*, though we constantly searched the wheat fields, berry bushes, &c.

If the food is properly placed in the cages, the bugs can be kept under constant observation. We have frequently watched them deposit their eggs, and were able to note approximately the length of time of each mating. The number of bugs in each cage must necessarily be limited, or accurate observation is impossible. As a rule we never placed more than four pairs in a cage, and as soon as a pair was found mating, they were transferred at once to a separate cage, in which they were kept isolated for the entire breeding season. Thus, for the important experiments, a single pair of bugs was confined in one cage, and each mating and the deposition of each batch of eggs could be accurately noted.

The cages suitable for the adult bugs are of course not suitable for the larval stages, unless a much finer wire mesh is used for the cover. After various experiments we found wet chamber dishes the most satisfactory

cage for the nymphs, care being taken to select a size deep enough for the food and large enough to hold from 20 to 30 nymphs without overcrowding. Sometimes as many as 30 nymphs are hatched from a single batch of eggs, and these may be very active after the first moult. We selected wet chamber dishes, about 120 mm. in diameter and 25 mm. deep. Experiments proved that the nymphs have sufficient air in these dishes, and can be raised with safety through the five moults to the winged stage.

The food suitable for the adult bugs is not satisfactory for the larval stages. Until after the third moult the nymphs are so small that they hide under the petals of the berries and in the grasses, and it requires too much time and patience to find them. The most satisfactory food for these stages proved to be the small, tender leaves that form the centre of headed lettuce. Experiment showed that the nymphs often select these leaves in preference to the berries, and they certainly develop most satisfactorily on this food, through the five moults to the winged stage. During the height of the breeding season we used 15 heads of lettuce a day, as the nymphs were transferred to a clean cage and fresh lettuce every other day. In making this transfer the cage containing the nymphs was carried to a separate table, used only for this purpose. The clean cage, labelled and containing fresh lettuce leaves, was placed on the same table, and the nymphs were carefully lifted from each stale leaf of lettuce to the fresh cage by using a very fine camel's hair brush. The nymphs were carefully counted while moving them from one cage to another, and it very rarely happened that a nymph was accidentally thrown away with the discarded lettuce.

To keep the proper degree of moisture in the cages proved to be a very troublesome problem. A certain amount of moisture is necessary to preserve the food, but if the cotton which is wrapped around the stems of the wheat, &c., is too wet, some of the water may drop on to the glass bottom of the cage, and if a bug falls on its back in even a few drops of water, it frequently cannot regain its normal position, and may die in a few hours. This danger must be controlled, for bugs frequently drop from the top or sides of the cage, and from the food. Too much moisture is even more dangerous for the larval stages. The drops that collect on the inner surface of the glass cover of the wet chamber dishes and fall to the bottom of the dish, mean death to any nymphs that may fall on their backs into even one drop of moisture. It requires constant vigilance to avoid this danger, by frequently wiping off the moisture that collects on the inside of the glass cover. We tried to avoid this risk by placing ordinary filter-paper on the bottom of the cages, but this did not work well, for the tarsi of the bugs frequently caught in the fibres of this coarse paper. But by substituting the German hardened filter-paper for the coarser paper, we got rid of this difficulty. The filter-paper must be cut to fit the bottom of the cages exactly, and it must be kept moist, but not too wet, for too much moisture will rot the delicate lettuce leaves. We have frequently watched the adult bugs, and

also the nymphs, suck the water from this filter-paper, but apparently with no injurious after-effects. We aimed to keep the temperature at 80° Fahr. night and day, and we succeeded in keeping the heat quite constantly at this point by using an electric stove.

It requires unremitting care to raise these Hemiptera in the laboratory. They not only require constant watching during the day, but must be examined two or three times in the night. Not only is this necessary for accurate observations, but if the adults or nymphs are found on their backs, they can be turned over with a camel's hair brush and their lives thus saved.

All our records have been kept with the utmost care. We have recorded not only the number and date of the deposition of the eggs, but the date of hatching, the number hatched in each group, the date when each of the five moults occurred, and a record of just how many young survived each moult. This is very important, in order to know the exact number of nymphs in each wet chamber dish, and thus avoid the danger of unwittingly throwing away a nymph with the stale food. As a rule, the nymphs from a single batch of eggs were kept separate, but late in the season, when only a few nymphs hatched out from a group of eggs, these nymphs, after the 1st or 2nd moult, were added to a cage that contained other nymphs from the same parents. When possible the date and hour of the deposition of each batch of eggs was recorded, though this of course was only possible where the deposition of the eggs was actually observed. In all other cases the time given is only approximate; but as the food in the cages was arranged to expose to view the places generally selected by the bugs for depositing their eggs, they were not often overlooked, until the food was changed and a closer search was possible. It is very important to secure the eggs as soon as possible after they are laid, for we found that both the male and the female parents will occasionally suck the eggs. Sometimes the male and sometimes the female was found with the proboscis buried in one of the eggs, very busy sucking out the entire contents. They pass from one egg to the next and may destroy a large number of eggs, leaving only transparent empty shells.

Two sets of records were kept for each pair of bugs, one set recording the history of the parents, and the second set recording the development of their offspring.

A full copy of these notes would make too voluminous a record to be published here, but in order to compare the breeding habits of *variolarius*, *servus*, the crosses and the F₁ hybrids, we shall give a condensed extract from these notes, showing for some individual cases, the number of eggs deposited by one female, the relative number that hatched, and the relation between mating and the deposition of eggs. Records I.-XI. pp. 362-70.

We have frequently watched the hatching of the eggs and the subsequent five moults of the nymphs before they reach the winged stage. Nymphs from the same group of eggs that hatched the same day, or even the same

hour, grow very unequally, occasionally a few of them being so retarded that they have reached only the third moult at the time the others have completed the fifth moult. Nymphs showing such greatly retarded development almost invariably die.

DETAILS OF CROSSING *Euschistus variolarius* ♀ × *Euschistus servus* ♂.

In November 1911, we placed in the same cage three female *variolarius* and five male *servus*, all of which had recently passed the fifth moult. These eight specimens were kept together throughout the following winter. In the spring (May 3rd) one of these *variolarius* females mated with one of the male *servus*, this demonstrating the possibility of crossing these two species. This female *variolarius* was hatched in our laboratory September 11th, 1911. The month before we had collected a few wild specimens of *variolarius* in Ridgefield, Connecticut, in order to secure young, vigorous bugs to carry through the winter for our crossing experiments, and eggs were deposited several times in this cage. It was from one of these groups of eggs that we secured the three females we used for crossing with *servus*. There were 17 eggs in this group, but only seven of them hatched, this being due, undoubtedly, to their having been deposited so late in the season. Six of these seven nymphs—2 males and 4 females—were reared to the winged stage, the 5th moult not being completed until October 16th. Three of these four *variolarius* females were selected to cage with *servus*, and the two *variolarius* males were killed the fourth day after the fifth moult, and preserved in alcohol, in order to avoid the possibility of any question being raised as to the identification of the females as pure *variolarius*. One of these two males is shown on Pl. 28, photo 6, and the black genital spot, distinctive of the species *variolarius*, is clearly shown. We believe we gained a great advantage by selecting bugs that hatched out so late in the season, for females which reach the winged stage so late as the middle of October are sure to remain all winter as vigorous sexually immature bugs, thus offering the best possible chance for successful breeding experiments in the spring.

The five male *servus* that were kept through the winter with the three female *variolarius*, were hatched from eggs deposited in our laboratory by a female *servus* received from North Carolina the day before the eggs were laid. A group of 10 eggs was deposited September 17th; 9 of these hatched and 8 were reared to the winged stage, the 5th moult being completed October 28th. Five of these bugs were males, and three females. On November 3rd the five males were added to the cage which contained the three above-mentioned female *variolarius*. In the further description of this cross, this cage will be designated as Cage 2. None of these bugs mated until May 3rd when, as stated above, one pair mated 7 hours (see Record III. p. 364).

As none of these bugs had mated again by May 26th, and the males seemed less vigorous than some wild specimens we had received from North Carolina two days before, we decided to take the five male *servus* from Cage 2, and replace them with three of the wild specimens just received from North Carolina. Three of the five male *servus* that were taken from Cage 2 at this time are shown on Pl. 28. photo 5, and as all five came from the same batch of eggs, these three specimens will serve to demonstrate the species. Each of the three males selected from the wild specimens (to replace the five that were raised in the laboratory) had just mated for several hours with its own species. One was put in Cage 2 May 26th, one on May 27th, and one on May 28th. The next morning at 5 o'clock a pair was found mating, but they separated before we could isolate them. Later in the same day the same female and, probably, the same male mated again. They were then isolated by being left in Cage 2, while the remaining bugs were transferred to another cage. They were kept isolated in Cage 2 until the female died July 16th.

Before the breeding season commenced, one of the three original female *variolarius* had unfortunately been thrown away with the stale food, and we were therefore left with only two of the three original females. One of these was undersize and never mated, though she was in a cage for nearly a month with several wild male *servus*.

We succeeded therefore in crossing only one female *variolarius* with *servus*; but this proved to be a fortunate circumstance, for this pair was sufficiently fertile to give us all the F₁ and F₂ hybrids that we could possibly care for, as at the same time we were raising hybrids from a cross between *E. variolarius* ♀ and *E. ictericus* ♂.

The female *variolarius* and the male *servus* in Cage 2 mated again June 11th: they mated 51 hours. This was the last time this pair mated, though the female deposited eggs six times after this mating, while normally eggs are deposited only once or twice between two matings.

In order to compare the breeding habits of this cross and of the F₁ hybrids, with the breeding habits of pure *variolarius* and *servus* we will give a brief summary of the data we have collected from our laboratory experiments.

A more detailed account of some of these experiments is given on pp. 362-70, where we have reproduced extracts from our records of eleven pairs of bugs that were isolated through their entire breeding period. All our experiments with *variolarius* and with *servus* indicate that they are alike in certain details of their breeding habits. Records I. & II., pp. 362-3, give what we believe to be the normal breeding habits of both these species. These records show the approximate number of eggs deposited by one female during the breeding season, the approximate intervals of time between the deposition of eggs, the frequency of the matings, and their approximate duration. They show

that mating occurs during the breeding season at rather definite intervals in relation to the deposition of eggs, as a rule eggs being deposited only once or twice between two matings. These records show further that nearly all the eggs deposited during the height of the breeding season develop and hatch. The features in which the breeding habits of the crosses differ from the normal can be best appreciated by comparing the following brief summary of the results from the eleven records given on pp. 362-70.

RECORD I. (p. 362). *E. variolarius* (one pair).—210 eggs were deposited from May 22nd to August 25th. The pair mated 13 times, and eggs were deposited 9 times, but not oftener than once or twice between two matings. None of the eggs deposited after July 25th were kept. Of the 129 eggs deposited before that date all hatched except seven, which were undoubtedly injured by the male, for he was discovered sucking them almost immediately after they had been deposited. He had taken the entire contents from two of these eggs, leaving nothing but the transparent shells.

RECORD II. (p. 363). *E. variolarius* (one pair).—78 eggs were deposited between July 2nd and July 26th. The pair mated 12 times, and eggs were deposited 6 times,—in one instance being deposited 3 times between two matings. None of the eggs deposited after July 10th were kept. Of the 63 deposited before that date all hatched but three. This pair was killed July 26th.

The two records (I. & II.) show what we believe to be the normal breeding habit of both *E. variolarius* and *E. servus*. They show that nearly all eggs develop that are deposited during the height of the breeding season, and that mating occurs oftener than eggs are deposited. They show further that, as a rule, eggs are deposited not oftener than once or twice between two matings. In these three features the crosses vary greatly from the normal, for a very small percentage of the hybrid eggs develop, and mating is very rare. They are, however, quite normal as to the number of eggs deposited, and the intervals of their deposition. The difference between normal breeding and the cross, is shown by the following summary of Record III.

RECORD III. (p. 364). *E. variolarius* ♀ × *E. servus* ♂.—120 eggs were deposited from May 23rd to July 9th. This is quite the normal number, but an abnormally small proportion of these eggs developed. 83 showed no signs of any development, and were probably unfertilized. Five showed the initial stages of development, but failed to hatch, and only 32 of the 120 eggs developed to the point of hatching. Further, the deposition of many of these eggs was abnormal. They were frequently deposited in small irregular groups, such as those deposited by isolated females that have never mated.

For more than a month during the height of the breeding season—

June 11th to July 16th—this pair did not mate, and during this period eggs were deposited six times, whereas normally eggs are deposited only once or twice between two matings. This female deposited eggs 20 times, and mated only 4 times, whereas normally mating occurs more frequently than the deposition of eggs. These facts suggest that a more normal proportion of the hybrid eggs might have been developed if the matings had been normal. Whatever condition exists that interferes with the mating of these two species, it is evidently not due to the fact that either the male or the female was functionally inactive, for the female deposited the normal number of eggs at normal intervals, and the male, when temporarily transferred July 2nd to a cage containing three female *servus*, mated the same day with one of his own species, and continued to mate for 42 hours. Further, he had mated with his own species before being caged with the female *variolarius*. July 2nd, we placed a few fresh wild *servus* males in Cage 2, but the *variolarius* female did not mate again, and died, July 16th.

Of the 32 F₁ hybrid nymphs from this pair, 27 were reared to the winged stage (11 ♂ & 16 ♀). We have photographs of all these eleven males, and ten of them are reproduced on Plate 28, photos 7 to 14.

There is a marked similarity between the abnormal features of this cross and those of a cross between an F₁ hybrid female and a pure *variolarius* male. To compare the breeding of these two crosses we will give, at this point, a brief summary of the results from Record XI.

RECORD XI. (p. 370). F₁ Hybrid ♀ × Pure *variolarius* ♂.—119 eggs were deposited between August 25th and September 18th. Only 36 of these hatched. Eggs were deposited 12 times and mating occurred only once. This pair did not mate a second time, though they were caged through their entire breeding period, which, in this case, did not begin until August 22nd*.

The two crosses of Records III. and XI. resemble each other in the fact that the mating of neither pair was normal, and that an abnormally small proportion of their eggs hatched. They are unlike, however, in that almost a normal proportion of the eggs of the cross of Record XI. showed the initial stages of development. The development, however, was obviously abnormal, and many of these eggs failed to hatch. Of the 36 eggs that hatched, 26 were reared to the winged stage (18 ♂ & 8 ♀). All the males were photographed, and are shown on Plate 34, photos 62-66. The pure *variolarius* male of this cross is shown on Plate 34, photo 58.

* In this connection it is interesting to note that an F₁ hybrid ♀ (from *variolarius* ♀ × *ictericus* ♂) that was caged with a pure *ictericus* ♂, mated normally through the entire breeding season. The female, however, had been kept through the winter, and they mated for the first time June 15th.

*F*₁ *Hybrids*.—Thirty-two of this generation were hatched (see Record III. p. 364) and 27 were safely reared through the five moults to the winged stage. They hatched between June 4th and July 9th, and reached the winged stage between July 8th and August 3rd.

As soon as they reached the winged stage (*i. e.* after the 5th moult) they were transferred to a large cage and closely watched. As each pair mated, they were isolated, while mating, to a separate cage, and kept isolated throughout the entire breeding period. The eggs from these *F*₁ pairs were placed in wet chamber dishes as soon as they were deposited, and the *F*₂ nymphs were carried through their entire development in these dishes, until they reached the winged stage, when they were killed and preserved in glycerine, as described on p. 371.

On July 9th and 10th, seven of the *F*₁ hybrid nymphs reached the winged stage (3 ♀ & 4 ♂). On July 19th two of these *F*₁ hybrid pairs mated, each pair being transferred at once to a separate cage while mating. They were kept thus isolated through their entire breeding season (see Records IV. and V., pp. 364-5). In this manner we isolated seven pairs of these *F*₁ hybrids.

Records IV. to X. give the details of these breeding experiments, and a brief summary of the results will show what features are typical of this generation of *F*₁ hybrids.

RECORD IV. (p. 364). *First Pair of F*₁ *Hybrids*.—169 eggs were deposited from August 2nd to August 31st. The pair mated 14 times, and eggs were deposited 7 times, and not oftener than once or twice between two matings. Six of the 169 eggs were killed for cytological study, and of the remaining 163, at least 154 hatched. (The number hatched is underestimated, as in two cases, only those nymphs were counted that survived the first moult.) 96 of these nymphs were reared to the winged stage (46 ♂ & 50 ♀).

Forty-three of the males were photographed, and are shown on Plate 29. Three of the males died in the cage just after the fifth moult. The ventral surface, including the genital segment of these three males, was dark and pathological, and of no value for the demonstration of the genital spot.

A photograph of the male of this pair of *F*₁ hybrids is shown on Plate 28, photo 9.

RECORD V. (p. 365). *Second Pair of F*₁ *Hybrids*.—184 eggs were deposited from August 1st to September 11th. The pair mated 25 times, and eggs were deposited 10 times, and not oftener than once or twice between two matings.

The female was discovered August 1st sucking the group of 28 eggs she had just deposited. She had sucked the entire contents from seven

of the eggs, and she must have injured others, as only eleven of the group developed.

Of the remaining 173 eggs deposited by this female 119 hatched. For some reason we were able to rear only a small proportion of these to the winged stage. We succeeded in rearing only 57 (31 ♂ & 26 ♀). Thirty of these males were photographed, and are shown on Plate 30. The male of this pair of F_1 hybrids died September 16th. The female was killed September 21st, ten days after the last deposition of eggs.

See photo 14, Plate 28, for the male of this pair.

RECORD VI. (p. 366). *Third Pair of F_1 Hybrids.*—194 eggs were deposited from July 30th to September 2nd. The pair mated 21 times and eggs were deposited 10 times, and not oftener than once or twice between two matings. Seven of the 194 eggs were killed for cytological study. Of the remaining 187 at least 172 hatched—for the number hatched is underestimated, as in two cases we counted only those nymphs that survived the first moult.

110 of these nymphs were reared to the winged stage (54 ♂ & 56 ♀). Forty-eight of the males were photographed and are shown on Plate 31. Six males died just after the fifth moult. Two of these are preserved as pinned specimens, and four were destroyed because the ventral surface was dark and the bugs were of no value for demonstrating the genital spot. The male of this pair of F_1 hybrids was photographed, and is shown on Plate 28, photo 10.

RECORD VII. (p. 367). *Fourth Pair of F_1 Hybrids.*—170 eggs were deposited from August 8th to September 8th. The pair mated 10 times, and eggs were deposited 8 times, and not oftener than once or twice between two matings. Seven of the 170 eggs were killed for cytological study, and of the remaining 163 at least 130 hatched. This number is an underestimate, for in three cases we counted only those nymphs that survived the first moult. We succeeded in raising to the winged stage only 63 of the 130 nymphs that hatched (28 ♂ & 35 ♀). Twenty-seven of these males were photographed and are shown on Plate 32, photos 42-48.

We were forced to kill this pair September 11th, as we had as many nymphs in the laboratory as we could properly care for. See photo 12, Plate 28, for the male of this F_1 hybrid pair.

RECORD VIII. (p. 368). *Fifth Pair of F_1 Hybrids.*—110 eggs were deposited from August 8th to September 3rd. The pair mated 10 times and eggs were deposited 6 times, and not oftener than once between two matings. Only 29 of the 110 eggs hatched, and only 16 of these survived to the winged stage (4 ♂ & 12 ♀). The four males were photographed, and are shown on Plate 28, photos 15 and 16. September 3rd, both male and female of this

pair were killed. We were forced to discard some of the hybrids, and selected this pair because the small percentage of eggs that hatched indicated that they were not functioning normally in spite of the fact that the number of eggs deposited in relation to the number of matings was quite normal. The abnormally large percentage of eggs that failed to hatch may bear some relation to the fact that this female had probably deposited unfertilized eggs before mating, for she was one of two females in a cage in which unfertilized eggs had been deposited.

See photo 11, Plate 28, for the male of this F_1 hybrid pair.

Before giving a summary of the breeding results of the 6th and 7th pairs of F_1 hybrids (Records IX. & X., p. 369) we must give a brief account of some preliminary experiments.

Two F_1 hybrid females and three F_1 hybrid males* were put in Cage 34 immediately after they had reached the winged stage (between July 9th and 12th). By August 6th none of these bugs had mated, and a group of four unfertilized eggs had been deposited by one of these females.

The two females were then transferred to Cage 36, in which there were 4 F_1 hybrid females and 4 F_1 hybrid males that had not yet mated. At 2 P.M. of the same day two pairs were found mating, and were transferred, while mating, to separate cages (Nos. 43 & 44). We believe these two females were the two that were transferred to this cage from Cage 34, though we have no proof of this. One of these pairs (Cage 43) mated 45½ hours, and on August 12th mated again 6 hours. On August 15th the female died without having deposited any eggs. The second pair (Cage 44) is the Fifth pair of F_1 hybrids described above (Record VIII.).

Cage 36 now contained 4 females and 2 males that had never mated. The two males resembled bugs that are found in the fall after the breeding season: the ventral surface had become hard and grey, instead of a fresh green colour, which is typical at the breeding period.

On August 15th we added to this cage the male that had mated August 12th in Cage 43. Ten minutes after this male was put into Cage 36 he mated with one of the four females, and the pair was transferred, while mating, to Cage 46. They mated 15½ hours. There now remained in Cage 36 the three females and the two males with grey venter, none of which had mated. One of these females, however, had a fresh green venter and showed other signs of functional activity.

August 16th, we removed the two males with grey venter from this cage (36) and added the male from Cage 46 that had just mated. At

* Two of these males were killed August 11th and the testes mounted for cytological study. The bugs were preserved and are shown on Plate 28, photo 7. The third male was killed August 13th and preserved as a pinned specimen.

2 P.M. of the same day the female with the green venter mated with this male, and we transferred the pair to Cage 48. Thus the two females of Cages 46 & 48 were fertilized by the same male. The two grey venter males that had never mated were put back into Cage 36, which now contained these two males and two females, none of which had mated. On August 28th these four bugs were killed and preserved. The two males are shown on Plate 28, photo 8. The male that had mated with the two females (Cages 46 & 48 = Records IX. & X.) was transferred after each mating from one of these cages to the other during the rest of the breeding season. We hoped we could raise enough offspring from each of these two females for a comparative study of the transmission of the genital spot through two different females fertilized by the same male. We were, however, disappointed in this, as we succeeded in raising only seven males from one of the two females—not enough to be of value for comparative study.

The results, briefly, are as follows:—

RECORD IX. (p. 369). *Sixth Pair of F₁ Hybrids*.—134 eggs were deposited from August 22nd to September 19th. The pair mated 11 times, and eggs were deposited 10 times, and not oftener than once or twice between two matings. Only 33 of these 134 eggs developed to the point of hatching, although a great many more were fertilized, and there was no obvious reason why they did not hatch. Only 13 of these nymphs survived to the winged stage (7 ♂ & 6 ♀). Six of the males were photographed and are shown on Plate 32, photos 49 & 50. The male of this pair was killed September 19th and the female September 21st. The male was photographed and is shown on Plate 28, photo 13 (this male fertilized also the female of Record X.).

RECORD X. (p. 369). *Seventh Pair of F₁ Hybrids*.—120 eggs were deposited from August 20th to September 8th. The pair mated 8 times, and eggs were deposited 8 times, and in only one instance were they deposited more than twice between two matings.

104 of these 120 eggs hatched, and 68 of the nymphs were reared to the winged stage (34 ♂ & 34 ♀). Thirty-two of these males were photographed and are shown on Plate 33, photos 51–57. Two males died in the cage just after the 5th moult, and were destroyed because the ventral surface was dark and pathological.

The above summary of the records of the seven F₁ hybrid pairs shows several features that are apparently typical of this generation of hybrids. These records show further, that if the F₁ hybrids can be secured, the F₂ generation can be obtained in large numbers. The F₁ hybrid generation is quite as fertile as the original pairs of either pure *variolarius* or pure *servus*

during the height of the breeding season, and it is interesting to note that in this fertility they resemble the F_1 generation of *servus*, but not of *variolararius*, for in our experience we have only one case on record in which the F_1 generation of pure *variolararius* mated and deposited fertile eggs the same season. If these records (IV. to X.) are compared with Records I. and II. of pure *variolararius*, it will be seen that, although the F_1 hybrids are not quite normal as to the percentage of eggs that develop, they are entirely normal as to the relation between mating and deposition of eggs, for eggs are deposited only once or twice between two matings, and the matings far exceed in number the deposition of eggs.

The records show that towards the end of the breeding season mating became more frequent, in some cases the breeding season being closed by a series of matings of short duration, which continued several days after the last deposition of eggs. This we believe is characteristic of *servus*, and was typical also of the F_1 generation of two other species we received from the South—*E. ictericus* and *E. crassus*.

We realize our experiments have not been sufficiently numerous to warrant definite conclusions as to the breeding habits of the species we have studied, but they furnish reliable data as far as the limited number of experiments admit. The higher death-rate of the F_2 generation, both as to the eggs and nymphs, we believe was due in part to the fact that the weather was unseasonably cold, and a proper degree of temperature and moisture could not always be satisfactorily maintained for all the cages.

In order to repeat these experiments on a larger scale, a much more elaborate equipment should be available. The bugs should be kept in a hot-house where temperature and moisture can be properly regulated, and the lettuce used for food should be cultivated under supervision, to be sure that no insecticides are used in its cultivation. Further, a number of trained assistants is absolutely necessary. The material furnished by a single cross is at some period of the experiments more than two workers can properly care for. We were forced to cut short several important experiments on account of the impossibility of continuing satisfactorily the extra work they involved.

We believe that our success in being able to cross even one pair of *variolararius* and *servus* is due to the fact that the two females used for the experiments, hatched after the close of the breeding season, and were kept through the following winter. This belief is supported by the fact that we did not succeed in repeating the cross-breeding experiments during the summer with bugs of the first generation of that season, though we tried this with 16 female *variolararius* and 14 male *servus*. These experiments were carried on in three cages, the first started June 22nd, the second June 28th, and the third July 2nd. All the female *variolararius* had been raised in our laboratory during the early summer, and were transferred to these cages

immediately after reaching the winged stage. Five of the male *servus* used in these experiments were raised in our laboratory, and like the *variolarius* females, were transferred to the experiment cages immediately after reaching the winged stage. The other 9 males were wild specimens, received from North Carolina. These experiments were continued for nearly two months, and no mating occurred at any time. The experiments were not closed until many unfertilized eggs had been deposited in all the cages*.

Possibly nymphs captured in the field, and raised to maturity in the laboratory may be more easily bred from; but in our experience we have never been able to collect the wild nymphs early enough in the season to succeed in breeding them with each other, or with an alien species.

The reciprocal cross with the first generation (♀ *servus* & ♂ *variolarius*) also proved unsuccessful; these experiments, as in all other cases, being continued until unfertilized eggs had been deposited a number of times in each cage.

We believe our lack of success in these cross-breeding experiments was not wholly due to the fact that the males and females were of different species, for we were almost as unsuccessful in getting a second generation of pure *variolarius*, though we had much better success in raising the second generation from *servus*. The first generation of this species mated from 10 to 18 days after they reached the winged stage, and were very fertile.

Fortunately for the success of our cross-breeding experiments, the F₁ hybrids resembled *servus* and not *variolarius*, in that most of them mated readily in captivity, from 10 to 20 days after the last moult, and like the first generation of *servus* proved to be very fertile.

The following experiments show it is much more difficult to get a second generation from *variolarius* the same season, although the first generation, if kept through the winter, will normally mate and deposit eggs early in the spring.

In 1911 we experimented with a few pairs of young *variolarius*, all reared from the same batch of eggs. About twenty days after they had reached the winged stage, a few males and females were placed in the same cage, from August 6th to August 27th. During this period they did not mate once, though the females deposited unfertilized eggs, and dissection showed the males to be apparently sexually mature. Two females and four males from this same batch of eggs were carried through the following winter, and in

* As a rule unfertilized eggs are deposited quite differently from those that have been fertilized. The latter are deposited in flat, symmetrical groups containing sometimes more than 30 eggs, and all adhering together. Unfertilized eggs, on the contrary, are dropped here and there on leaves, grasses or berries, sometimes only one or two eggs at a time, or more frequently in groups of three, four, or five. We never destroyed the unfertilized eggs until ten days after their deposition, although fertilized eggs always show the initial stages of development on the 3rd or 4th day.

the spring they mated and deposited eggs quite normally; 330 eggs being deposited by the two females before July 9th, when they were killed. It was from these eggs that we raised the *variolarius* specimens used for the above described unsuccessful cross-breeding experiments with *servus*. We also tried to breed from several of this first generation of *variolarius* to use as a control for our cross-breeding experiments, and to test the above described experiment of 1911 with the first generation of that season. We had three cages of these experiments in 1912, including in all 18 females and 13 males. The first cage was started July 15th, and these experiments were not closed until August 29th. During this period only one pair* of these 31 bugs mated (August 16th) though many unfertilized eggs were deposited in the three cages. The fact that in all these experiments unfertilized eggs were deposited, proves that the young females function the same season, but our experiments indicate that the young males rarely function until the next spring. The following experiments bearing on this point may be added to those already given. As stated above, the young pairs of *variolarius* that were caged in 1911 did not mate, although the females deposited unfertilized eggs. Thinking this might be due to the fact that these bugs were all from the same batch of eggs, we caged one of these F₁ males with a wild female that had just mated with a wild male. They did not mate, however, although the female continued to deposit eggs at normal intervals until August 22nd, and was not killed until September 26th.

In the season of 1912 we were anxious to test this experiment by caging wild males, after they had mated in the laboratory, with young females that were depositing unfertilized eggs, but we did not succeed in capturing any *variolarius* males that season, though we searched ourselves, and had assistants searching also.

That the young *variolarius* males rarely function the same season in the laboratory was again indicated by the following experiment. Five young *variolarius* males had been caged for five weeks with five F₁ hybrid females, and had not mated once, although the females had deposited 88 unfertilized eggs. Thinking that perhaps these males might be sexually immature, they were replaced by the one young *variolarius* male that had mated in the laboratory that season. Eleven days later this male mated with one of the F₁ hybrid females (see Record XI. and p. 345).

These facts would seem to indicate that the young male *variolarius* are not as a rule sexually mature the same season they are hatched, but the evidence on this point is entirely inadequate as proof. *Variolarius* females that were depositing unfertilized eggs also failed to mate with the F₁ *servus* males, and these males were undoubtedly sexually mature, for they breed readily

* This pair was transferred to a separate cage while mating, and used for experiments described below. The male of this pair is shown on Plate 34, photo 58.

with their own species. This may indicate that possibly the factor of selection may be in part responsible for some of the failures of our breeding experiments.

We raised only 10 ♂ and 22 ♀ from the one pair of young *variolarius* that mated in the laboratory in 1912. We undoubtedly would have had more, but the female was fertilized only once, as we transferred this male to the F₁ hybrid females, as described above. This *variolarius* female deposited 58 eggs from August 17th to September 7th, and 36 of these hatched, 32 being reared to the winged stage (10 ♂ & 22 ♀). The F₁ hybrid female that was fertilized by this same male deposited 119 eggs and 36 hatched.

The males from these two females were photographed and are shown on Plate 34. Photos 59 to 61 show the males from the pure *variolarious* pair, and photos 61 to 66 show the males from the F₁ hybrid female and the pure *variolarius* male.

These photographs demonstrate that the spot is inherited through the pure *variolarius* female (photos 59 to 61) more intensely than it is through the F₁ hybrid female (photos 62 to 66), and a comparison of photos 62 to 66 with those when both parents are F₁ hybrids, Plates 29-33, demonstrates that the spot is transmitted through a pure male *variolarius* much more strongly than through an F₁ hybrid male. All such facts bearing on the inheritance of the genital spot are important in testing modern chromosome theories of sex-determination in the light of the transmission of this exclusively male character.

DISCUSSION.

Any analysis of the results of cross-breeding experiments involves a discussion of their bearing on fundamental problems of heredity, and we should examine the facts demonstrated by our recent experiments in the light of the popular theories which claim to offer a partial solution of some of the important problems of heredity. The hypothesis of first interest to the cytologist is the one that claims to offer an explanation of the transmission of characters by the assumption that the factors essential to their transmission are carried and distributed by definite chromosomes, but a discussion of our results from this point of view is reserved for a paper in which the cytological phenomena can be fully demonstrated by photographs. A brief statement of the facts and their bearing on recent chromosome theories was given in the preliminary report of our work ('13).

In the present paper we shall merely restate the facts and conclusions in order to present the evidence in detail, as it is demonstrated in the photographs of Plates 28-34.

First. The results demonstrate that an exclusively male character (the genital spot) can be inherited without the aid of the Y chromosome. This is

proved by the fact that it is transmitted through the female, and the female does not have the Y chromosome, as this chromosome is an exclusively male character. Photographs 7-57 show the males of the F_1 and the F_2 generations. All these males are the hybrid descendants of one pure *variolarius* female, that was fertilized by *servus*—the species that has no genital spot; and these hybrids show beyond question that the spot can be transmitted through the female, some of the F_2 males having as pronounced a spot on the genital segment as that of the *variolarius* males: *e. g.*, one or more of the specimens of photos 15, 23, 26, 28, 32, 34, 35, 36, 40, 41, 46, 48, 55.

Second. The results demonstrate that the genital spot can be inherited without the aid of the X chromosome. This is proved by the fact that it is transmitted through the male, and the male-producing spermatozoon does not have an X chromosome. Photos 62 to 66 show the males from an F_1 hybrid ♀ × a pure *variolarius* ♂, and a comparison of these photographs with those of the F_2 hybrid generation (photos 15 to 57) shows that the genital spot is inherited much more strongly from the pure *variolarius* male than through the F_1 hybrid males; this fact demonstrating that the male *variolarius*, as well as the female *variolarius*, can directly transmit the spot to the males. As, according to the hypothesis, these can be inherited directly from the male, only through the male-producing spermatozoon, which has no X chromosome: it follows that the spot can be inherited without the aid of the X chromosome. This back cross further demonstrates, not only the direct inheritance of the spot from the male, but also the inheritance of the *servus* character, absence of spot. This was transmitted to the F_1 ♀ by the pure *variolarius* ♀ of the first cross, and therefore *ex hypothesi* it must have come from the female-producing spermatozoon of *servus*. This back cross therefore demonstrates that an exclusively male character—the genital spot—can be transmitted by the male-producing spermatozoon, and an exclusively male character—the absence of spot—can be transmitted by the female-producing spermatozoon, and that therefore these so-called sex-determining spermatozoa do not differ functionally in their transmission of an exclusively male character. In making these deductions, it is, of course, necessary to accept, for the sake of the argument, the assumption of male- and female-producing spermatozoa, an assumption which, we believe, is far from being proved.

Third. The results show that if we assume that the factors necessary for the production of the genital spot are located in any of the ordinary chromosomes, they must be in at least both members of a pair of ordinary chromosomes, for the spot is directly transmitted through both the male and the female.

Fourth. The results show that if we assume that the factors necessary for the production of the genital spot are carried by both members of a pair of chromosomes, we must assume that the female carries an inhibitor for the

spot, as the spot is never present in any of the females, neither in the pure *variolarius* nor in the hybrids, although the fact that it is transmitted by the female *variolarius* proves that the spot factors are present in the female, though not expressed.

Fifth. The results show that, although it is necessary to assume an inhibitor only in the females of pure *variolarius*, in the hybrids it becomes necessary to assume an inhibitor in the males also. The F_1 hybrid males show the spot either very incompletely, or not at all, although they can transmit the spot to the next generation, and therefore they must carry the factors necessary for its transmission, in spite of the fact that the appearance of the spot in the F_1 generation is partly or wholly inhibited. The two F_1 hybrid males of photo 7 have no spot*, the upper F_1 male of photo 8 has merely an indication of a spot and in the lower bug it is not much stronger. The F_1 males of photos 9 to 13 have a very insignificant spot, and the F_1 male of photo 14 has merely a trace of a spot, although the offspring of these males frequently have a spot quite as pronounced as that of pure *variolarius*. Compare these F_1 males with one or more of the F_2 males of photos 15, 23, 26, 28, 32, 34, 35, 36, 40, 41, 46, 48, and 55.

Sixth. The facts show that if we attempt to place this inhibitor in definite chromosomes, we meet as serious difficulties as those involved in assuming that the factors essential for the production of the genital spot are carried by special chromosomes. In our preliminary report of these experiments (13), we discussed in full the evident results of placing this inhibitor in various chromosomes: in the X chromosomes, in one of the ordinary chromosomes, or in a pair of chromosomes, and we found that none of these assumptions would accord with the facts. The facts force us to regard these inhibitors as hypothetical forces which cannot logically be confined to the chromosomes, and are located we know not where—these hypothetical inhibitors practically doing work that has been assigned to definite chromosomes. As stated in the above-mentioned preliminary report, "the facts force us to consign to these hypothetical inhibitors, not only the responsibility of suppressing the spot factors in all the females, but also of determining just how many spot factors shall find expression in the males of the F_1 and F_2 generations, and thus they practically relieve the chromosomes of the burden of unit distribution."

It would seem then that the facts are out of harmony with the theories that offer an explanation of the transmission of characters on the assumption that the factors essential to their transmission are carried and distributed by definite chromosomes.

* The two dark specks on the genital segment of the upper bug, close to the base of the segment, must not be confused with the genital spot. In the lower bug a slight indication of a spot can be seen with a lens, but it will probably not appear in the print.

If we examine the results of our experiments in the light of Mendel's law of heredity, we find that the genital spot does not behave as a Mendelian unit. Professor Punnett (1911) defines a unit-character as follows: "Unit-characters are represented by definite factors in the gamete which, in the process of heredity, behave as indivisible entities, and are distributed according to a definite scheme. The factor for this or that unit-character is either present in the gamete, or it is not present. It must be there in its entirety, or be completely absent." (Page 42.)

Whatever determines the genital spot in these hybrids, it cannot be an indivisible unit-factor, which is "present in its entirety" or is "completely absent," for the genital spot is not present as a whole, or completely absent. If we speak of it in terms of Mendelism, we must say that it is the result of a number of unit-factors, for in the hybrid males of the F_1 and F_2 generations in which a spot can be identified, it is present in every degree of intensity, from a mere indication of a spot, to that of the F_2 generation which is quite as conspicuous as the spot of a pure *variolarius*.

Neither the spot nor its absence is dominant in the F_1 hybrids. There is certainly an absence of dominance in this F_1 generation. Of the eleven F_1 male hybrids, 2 have no spot (photo 7): 4 have a spot so faint that it is barely visible (photos 8 and 14 and one pinned specimen); and 5 have a spot about one third as pronounced as that of a pure *variolarius* (photos 9, 10, 11, 12, and 13).

It is evident also that the spot of these F_1 hybrids is not a true blend, for only one parent is represented by the two specimens that have no spot, and there is quite as much variation in the size and intensity of the spot of the remaining nine specimens as is found in any nine intermediates of the F_2 generation.

The F_2 generation shows apparently a greater variability than the F_1 generation, for in the former *both* extremes are represented, some of the males having a spot as pronounced as that of pure *variolarius*, while some have no spot whatever, and the remainder have the spot in varying degrees of size and intensity. These facts may be of value as offering a test such as Castle (1911) has suggested, by which it may be possible to decide whether the results can be classed with the Mendelian type of inheritance, or with that described as a "non-Mendelian, non-segregating type of inheritance." Castle says: "There is one means by which we can determine with certainty whether, in a particular case of seemingly blending inheritance, segregation does or does not occur, namely by comparing the variability of the F_1 and F_2 generations. If segregation does not occur, F_2 should be no more variable than F_1 , whereas if segregation does occur, F_2 should be more variable." (Page 137.)

Although the F_2 generation of our *Euschistus* hybrids shows more variability than the F_1 generation, the value of this as proof is weakened, if not

cancelled, by the fact that the two generations are not equally represented, and cannot therefore be justly compared. We have 190 males of the F_2 generation in which the exact condition of the spot can be determined, and only 11 males of the F_1 generation; and therefore it would not be reasonable to conclude from such inadequate data, that the F_1 generation of these hybrids is less variable than the F_2 generation. We are inclined to believe that if the number of the F_1 hybrid males were equal to that of the F_2 generation, they would show both extremes of the inheritance of the spot, and a variability quite as pronounced as we find in the F_2 males.

Some of the males of the F_2 generation might be assumed to be pure dominants and recessives, for some have the spot as fully developed as that of pure *variolarius* (one or more of the specimens of photos 15, 23, 26, 28, 32, 34, 36, 40, 41, 46, 48, and 55), while others have no spot whatever (one or more of the specimens of photos 16 to 39, 43, and 51 to 56). The intermediates show the spot in a very variable degree of intensity, but if these latter are assumed to be "visible heterozygous forms" of Mendelian segregation, there should be an equal number of pure dominants and recessives, whereas there are only 19 that have the perfect *variolarius* spot, and 74 in which the spot is absent.

If we would attempt to find a theoretical explanation of the results, we must first devise a formula that will work for pure *variolarius*, and the facts compel two assumptions in the making of such a formula. First, we must assume that the female is homozygous for the spot factors, and second, that she has an inhibitor for the spot and that she does not transmit this inhibitor to her male offspring. The necessity for this last assumption seems obvious, because the spot is *never* inhibited in the male. If we would assume, however, that both sexes can have an inhibitor, an assumption that some facts in the hybrids demand, then we must assume that it requires a double dose to inhibit the spot; and to insure the spot being a constant character, we must assume that in the male the inhibitor is not only heterozygous but sex-linked. But this assumption, which would hold for *variolarius*, would not work for the cross, because the *female hybrids* would in this case be heterozygous instead of homozygous for the inhibitor, and would therefore have the spot. If to obviate this difficulty we assume that *servus* also has an inhibitor, this would inhibit the spot in the F_1 hybrid *females*, but in *none* of the F_1 hybrid *males*, whereas in fact, the spot is completely or partially inhibited in *all* the F_1 hybrid males.

The necessity of the assumption that the female *variolarius* is homozygous for the *spot factors* is quite obvious, for the genital spot could not remain constant if we assume that the female is heterozygous for the spot factors, unless we add the unwarrantable assumptions that the female is heterozygous and the male homozygous for sex, and that the spot factors are linked with the sex-factor.

We seem compelled, therefore, to assume that the female *variolarius* is homozygous for the spot factors; and if we assume that *servus* is without them, then the F_1 hybrids should be heterozygous for these factors. Assuming that they are inhibited in the female, the males should *all be alike*, either in having a *variolarius* spot or in having no spot. The facts, however, are as follows:—Two of the eleven F_1 hybrid males have no spot (photo 7), and the remaining nine are variable intermediates (8 of the 9 are shown in photos 8–14, and the 9th is a pinned specimen, which has a spot so small and so faint that it is scarcely perceptible).

An interesting case of F_1 hybrids approximating a blend is the cross between the pigmented silky hen and the unpigmented brown leghorn. Prof. Punnett theoretically explains these intermediates (partly pigmented) F_1 fowls by the following assumptions:—

- 1st. Assumes that the ♀ is heterozygous for femaleness (Ff).
- 2nd. Assumes that the ♂ is without this sex-factor (ff).
- 3rd. Assumes that the silky is homozygous for the pigment factor (PP).
- 4th. Assumes that the silky is without an inhibiting factor (ii).
- 5th. Assumes that the brown leghorn is without the pigment factor (pp).
- 6th. Assumes that the brown leghorn is homozygous for an inhibiting factor (II).
- 7th. Assumes the inhibitor is sex-linked (there is a repulsion between I and F).

If we transfer all these assumptions to the *variolarius* × *servus* cross, even to the extent of assuming that the female instead of the male is heterozygous for the sex factor, they fail to account for the fact that these F_1 hybrids are not *all* intermediates.* *Two are like servus in having no spot.* Such irregularities in the F_1 generation are out of harmony with the principles of Mendelism, but in some forms similar evidence against Mendelism has been weakened, if not cancelled, by the fact that the F_2 generation shows a regular Mendelian ratio. This is not the case, however, with the F_2 generation of the *Euschistus* cross—of the 190 F_2 male hybrids (photos 15–57), 19 have the spot quite as distinct as that of pure *variolarius*, 97 are very variable intermediates, and 74 are like *servus* in having no spot.

The classification of the F_2 hybrids into these three groups is based on a study of the photographs,† and the numbers in each group were afterwards

* It is obvious that in the *females* of *variolarius* a special inhibitor must be assumed that is outside the germ plasm—for *theoretically* the spot appears in the female.

† In classifying the photographs of the hybrids we included under the *servus* type not only all those specimens with no spot whatever, but also those with merely a faint indication of a spot, for the latter was not visible in the living specimens, and probably will not show in the reproductions.

compared with those based on a study of the original specimens immediately after the bugs were killed. The two sets of figures agree almost exactly, differing only in the fact that in the original estimate we classed two specimens as *variolarius* and two as *servus* which we now class as intermediates. Such a variation in the classification is unavoidable because the intermediates blend into the two extremes, and it is not always clear into which division a specimen belongs. Possibly the printing of the photographs in the final plates may be too light in some cases, and fail to bring out a faint spot that should be classed as an intermediate and not *servus*, and this would cause some slight variations from the above figures. If we consider separately the ratio from each of the seven pairs of F_1 hybrids, the results are as follows:—

F. 2 males from the seven pairs of F_1 hybrids.

| | 1st pair. Plate 29, Photos 17-25. | 2nd pair. Plate 30, Photos 26-32. | 3rd pair. Plate 31, Photos 33-41. | 4th pair. Plate 32, Photos 42-48. | 5th pair. Plate 28, Photos 15-16. | 6th pair. Plate 32, Photos 49-50. | 7th pair. Plate 33, Photos 51-57. | Totals. |
|--------------------------------------|--|--|--|--|--|--|--|---------|
| Spot like <i>variolarius</i> . | 1 | 3 | 8 | 4 | 1 | 0 | 2 | 19 |
| Inter- mediates. | 17 | 12 | 27 | 18 | 2 | 5 | 16 | 97 |
| No spot like <i>servus</i> . | 25 | 15 | 13 | 5 | 1 | 1 | 14 | 74 |

Of these seven pairs of F_1 hybrids only the 5th pair shows a simple Mendelian ratio (1 like *variolarius*, 2 intermediate, and 1 like *servus*), a ratio which evidently would not have been maintained if a large number of offspring had been secured, for no such ratio is shown by the F_2 hybrids from any of the remaining six pairs, all of which have a larger number of offspring. If one is willing to readjust these ratios by filling the gaps with the nymphs that died and those that failed to hatch, it would be possible of course to create any ratio required, but the assumptions cannot be the same for the seven pairs, and this fact would weaken the value of such an adjustment.

We may further search for a Mendelian ratio (half pure and half heterozygous) in the cross between the F_1 hybrid ♀ and the pure *variolarius* ♂. From this cross we raised 18 males (photos 62-66). 12 of these 18 males

have the spot as strong as *variolarius*, 3 have a spot so small and faint that it is scarcely perceptible (the lower two specimens of photo 62, and the lowest specimen of photo 66), and 3 are quite typical intermediates (the 3rd and 5th specimens of photo 64, and the second specimen of photo 66). Thus 12 of these males have the *variolarius* spot, 3 are intermediates, and 3 are almost without a spot. It therefore seems obvious that a Mendelian ratio is not shown in any of the experiments, it does not appear in the F_1 hybrid generation, in the F_2 hybrid generation, nor in this generation from an F_1 hybrid ♀ by pure *variolarius* ♂.

The above-mentioned cross (F_1 hybrid ♀ × pure *variolarius* ♂) is important because it shows that the genital spot is not a sex-linked character. The test of whether a character is linked to a factor that determines sex is usually made by the "reciprocal cross," which in this case would be pure *servus* ♀ × pure *variolarius* ♂. It is assumed that the parent that is heterozygous for sex can transmit a sex-linked character only to the opposite sex, and therefore none of the males from such a cross should inherit the spot. We were unable to attempt this cross, but the above-mentioned experiment (F_1 hybrid ♀ × pure *variolarius* ♂) shows quite as conclusively as a "reciprocal cross" whether the genital spot behaves as a sex-linked character. This cross demonstrates that the genital spot does *not* behave as a sex-linked character, for it shows that the male *variolarius* can *directly* transmit the spot to his male offspring, for these offspring show the greatly increased strength of its inheritance from the pure male as compared with its inheritance from the F_1 males.

We cannot explain this exclusively male character by simply assuming that the constitution of the eggs inhibits the expression of the spot in the female, for this leaves unexplained the fact that the spot is partly or wholly inhibited in the F_1 males.

We have been unable to harmonize our results either with the Mendelian or non-Mendelian (blend) type of inheritance—the great variability of the F_1 hybrids being the most obvious difficulty. The assumption of multiple factors may be satisfactory as an explanation of the variability of the F_2 intermediates; but it does not explain the variability of the F_1 hybrids. On the assumption of multiple factors the F_1 hybrids should be alike—subject only to minor variations (fluctuations). This, however, is not the case—2 have no spot and 9 are variable intermediates.

Castle's assumption of change in potency of a given factor or factors seems more in harmony with the facts, for this offers not only an explanation of the variability of the F_2 intermediates, but admits any degree of variability in the F_1 hybrids—even to the extent of reversing dominance.

We cannot logically explain the facts on the assumption of variation in potency of a single spot factor, for on this assumption the spot of pure *variolarius* should show the same relative amount of variation, which is not the case. But if we assume that *servus* has an inhibitor which also varies in potency, then the F_1 spot, besides being more or less reduced by a single dose of spot factor which varies in potency, can be further reduced or wholly cancelled by the *servus* inhibitor, according to the degree of its potency.

The hypothesis of varying potency of unit factors demands the presence of at least a perceptible variation in the spot of pure *variolarius*, and such a variability does, in fact, occur. This irregularity can be best appreciated by comparing the male offspring from the same parents.

Castle (1912) says: "In my experience every unit character is subject to quantitative variation, that is, its expression in the body varies." The fundamental explanation of his striking results in selection is the assumption that these variations have a germinal basis and are inherited.

It seems to us that our results are more in harmony with Castle's assumption of the varying potency of unit factors; but if they do in fact admit of a pure Mendelian interpretation, this must be left to the specialist in genetics.

APPENDIX I.

Since this paper was finished, striking corroborative support has been given to the results of our experiments.

In expressing to Professor Poulton our desire to find an experienced Entomologist who would look over the parent species of our hybrids for some distinguishing character (other than the genital spot) that might give additional evidence in support of our experiments, Professor Poulton kindly suggested Dr. Harry Eltringham, of New College, Oxford. We feel very grateful to Professor Poulton for his interest and courtesy in this matter, and we are deeply indebted to Dr. Eltringham for his very valuable discovery of the marked difference in the length of the intromittent organ of *E. variolarius* and *E. servus*. As a result of his observation, we have been able to follow out the inheritance of this second exclusively male character in the hybrids from this cross, as well as from the cross between *E. variolarius* and *E. ictericus*.

APPENDIX II.

RECORDS OF ELEVEN PAIRS OF BUGS THAT WERE ISOLATED
THROUGH THEIR BREEDING PERIOD.

As details of breeding experiments can be of value or interest only to those who may care to repeat such experiments with the same or allied species, we have not published the following details in the text, but have added them here in a convenient form for reference.

The following records are extracts from our notes. We have selected only the main points essential for comparison, omitting such details as the dates of hatching, the dates of the five moults, and the number of nymphs that survived each moult, &c., &c. The length of time given for each mating is the minimum, as it is estimated from the time the pair was found mating until the last record before they were found apart. The bugs were closely watched during the day, and observations made two or three times during the night. The number of eggs that hatched is also the minimum, for in some cases we counted only those nymphs that survived the first moult. Before the first moult these small bugs generally keep closely segregated in a compact mass, and it is impossible to count them accurately when a large number is hatched.

RECORD I. CAGE 2.—1911.

One Pair of *E. variolarius*.

(Collected April 16th, at White Plains, New York, by J. R. de la torre Bueno.)

APRIL

- 20th. 6.30 A.M. mating: continued to mate 9½ hours.
28th. 5.00 P.M. ditto ditto 19 hours.

MAY

- 2nd. 1.30 P.M. ditto ditto 17½ hours.
7th. 6.00 A.M. ditto ditto 9 hours.
10th. 6.00 A.M. ditto ditto 15 hours.

22nd. 4 eggs, all hatched: 3 reared to winged stage—(2 ♂ & 1 ♀).

JUNE

- 9th. 29 eggs, 20 hatched. (Found the ♂ sucking these eggs: had sucked the entire contents from two.) 16 reared to winged stage—(7 ♂ & 9 ♀).
26th. 5.30 A.M. mating: continued to mate 3½ hours.

JULY

- 1st. 28 eggs, all hatched: 15 reared to winged stage—(9 ♂ & 6 ♀).
7th. 28 eggs, all hatched. Killed all after 3rd moult to preserve as nymphs.
13th. 5.30 A.M. mating: continued to mate 6½ hours.
14th. 5.30 A.M. ditto ditto 4¼ hours.

JULY

- 20th. 5.00 A.M. 28 eggs, all hatched. Killed all after 1st moult to preserve as nymphs.
 20th. 5.00 A.M. mating: continued to mate 3 hours.
 23rd. 5.30 A.M. ditto ditto $5\frac{1}{2}$ hours.
 25th. 12 eggs, all hatched. Did not keep these nymphs beyond the 2nd moult.

AUGUST

- 5th. 42 eggs, in 3 groups. These eggs not kept.
 6th. 5.00 A.M. mating: continued to mate 4 hours.
 10th. 11 eggs. These eggs not kept.
 19th. 5.30 A.M. mating: continued to mate 27 hours.
 23rd. 5.30 A.M. ditto ditto $6\frac{1}{2}$ hours.
 25th. 28 eggs. These eggs not kept.
 31st. The female died. Killed the male and preserved the pair in alcohol.

RECORD II. CAGE 10.—1911.

One Pair of *E. variolarius*.

(Collected April 16th, at White Plains, New York, by J. R. de la torre Bueno.)

APRIL

- 18th. 7.00 A.M. mating: continued to mate 6 hours.
 22nd. 7.30 A.M. ditto ditto $8\frac{1}{2}$ hours.
 25th. 1.00 P.M. ditto ditto $5\frac{1}{2}$ hours.
 28th. 6.30 A.M. ditto ditto $4\frac{3}{4}$ hours.

MAY

- 1st. 6.30 A.M. ditto ditto $6\frac{3}{4}$ hours.
 10th. 6.00 A.M. ditto ditto $27\frac{1}{2}$ hours.
 23rd. 6.00 A.M. ditto ditto $33\frac{1}{2}$ hours.

JUNE

- 6th. 6.00 A.M. ditto ditto 14 hours.
 17th. 5.30 A.M. ditto ditto 14 hours.

JULY

- 2nd. 28 eggs (watched the ♀ depositing some of these eggs): 27 hatched.
 Nymphs not kept after 3rd moult.
 3rd. 5.30 A.M. mating: continued to mate 36 hours.
 5th. 14 eggs, 12 hatched. Killed after 2nd moult to preserve as nymphs.
 7th. 13 eggs, all hatched. Nymphs not kept after 2nd moult.
 10th. 8 eggs, all hatched. Nymphs not kept after 1st moult.
 12th. 5.30 A.M. mating: continued to mate 29 hours.
 16th. 3.30 P.M. ditto ditto $19\frac{1}{2}$ hours.
 20th. 10 eggs. These eggs not kept.
 26th. 5 eggs. ditto.
 26th. Killed both the ♂ & ♀ and preserved. We were forced to kill this pair as at this period we had as much living material in our laboratory as we could properly care for.

RECORD III. CAGE 2.—1912.

E. variolarius ♀ × *E. servus* ♂.

MAY

- 3rd. 3.00 P.M. mating : continued to mate 7 hours.
 23rd. 1 egg, unfertilized (did not show the initial stages of development).
 27th. 7 eggs, ditto ditto ditto
 28th. 6 eggs : one developed and hatched.
 29th. 5.00 A.M. mating : continued to mate 15 minutes.
 29th. 1.15 P.M. ditto ditto 18 hours.

JUNE

- 2nd. 17 eggs : 3 apparently unfertilized, 14 hatched.
 8th. 5 eggs : 3 apparently unfertilized, 2 hatched.
 11th. 18 eggs, in 3 groups : 12 apparently unfertilized, 6 hatched.
 11th. 3.50 P.M. mating : continued to mate 51 hours.
 14th. 13 eggs, in 2 groups : 4 apparently unfertilized, 9 fertilized, 8 of which hatched.
 18th. 8 eggs : 7 apparently unfertilized ; 1 fertilized, but did not hatch. (Watched the female depositing some of these eggs.)
 21st. 10 eggs : 9 apparently unfertilized ; 1 fertilized, but did not hatch. (Watched the female depositing some of these eggs.)
 25th. 10 eggs, in 2 groups : 8 apparently unfertilized, 2 hatched. (Watched the female depositing some of these eggs.)
 30th. 16 eggs, in 3 separate groups : 15 apparently unfertilized, 1 hatched.

JULY

- 9th. 9 eggs, in 3 groups : all apparently unfertilized.
 16th. The female died.
 Total number of eggs deposited..... 120
 " " apparently not fertilized..... 83
 " " fertilized but failed to hatch.. 5
 " " hatched 32

RECORD IV. CAGE 38.—1912.

First Pair of F₁ Hybrids.

JULY

- 19th. 6.00 P.M. mating : continued to mate 15 hours.
 28th. 5.00 A.M. ditto ditto 28 hours.

AUGUST

- 2nd. 31 eggs *, 30 hatched : 14 reared to the winged stage—(9 ♂ & 5 ♀).
 3rd. 5.00 A.M. mating : continued to mate 16 hours.
 4th. 17 eggs, all hatched : 13 reared to the winged stage—(5 ♂ & 8 ♀).
 7th. 5.30 P.M. mating : continued to mate 15½ hours
 9th. 22 eggs, all hatched : 13 reared to the winged stage—(6 ♂ & 7 ♀).
 12th. 28 eggs : 26 survived 1st moult : 18 reared to the winged stage—(9 ♂ & 9 ♀).

* Unless stated to the contrary, the eggs were deposited in one symmetrical group, which is typical of both *variolarius* and *servus*.

AUGUST

- 13th. 5.00 A.M. mating: continued to mate 26 hours.
 17th. 9.00 P.M. ditto ditto 14 hours.
 19th. 30 eggs (one group of 8 & one group of 22 eggs): killed 6 for cytological study.
 14 reared to winged stage—(9 ♂ & 5 ♀).
 19th. 6.00 P.M. mating: continued to mate 26 hours.
 21st. 7.30 P.M. ditto ditto 2 hours.
 22nd. 3.30 P.M. ditto ditto 5 hours.
 24th. 5.45 P.M. ditto ditto 5 hours.
 25th. 6.30 P.M. ditto ditto 4 hours.
 26th. 5.30 P.M. ditto ditto 4½ hours.
 27th. 21 eggs (at 10 A.M.): 18 survived 1st moult, 12 reared to winged stage—
 (3 ♂ & 9 ♀).
 27th. 5.30 P.M. mating: continued to mate 3 hours.
 28th. 4.00 P.M. ditto ditto 3½ hours.

JULY

- 30th. The male died and was preserved in glycerine (tube 34).
 31st. 20 eggs (deposited on wire top of cage): 19 hatched, 12 reared to winged stage—(5 ♂ & 7 ♀).

SEPTEMBER

- 3rd. Killed the ♀ and preserved in glycerine. This female would undoubtedly have deposited more fertilized eggs, but at this period we had as many nymphs developing from all the hybrid pairs as we could properly care for, and were forced to be satisfied with the number of offspring already secured from this pair. The male was photographed, and is shown on Plate 28, photo 9.

RECORD V. CAGE 39.—1912.

Second Pair of F₁ Hybrids.

JULY

- 19th. 9.00 P.M. mating: continued to mate 13 hours.
 30th. 5.00 P.M. ditto ditto 16½ hours.

AUGUST

- 1st. 28 eggs. Discovered the ♀ sucking these eggs; she had taken the entire contents from 7 and probably more were injured, as only 11 hatched.
 8 reared to winged stage—(5 ♂ & 3 ♀).
 5th. 28 eggs, 17 hatched (6 survived 2nd moult). These were later caged with nymphs from eggs deposited August 12th.
 6th. 6.00 P.M. mating: continued to mate 57 hours.
 12th. 11 eggs, 3 hatched. These were later caged with nymphs from eggs deposited August 5th. 8 reared to winged stage—(4 ♂ & 4 ♀).
 12th. 7.30 P.M. mating: continued to mate 12½ hours.
 13th. 30 eggs, 24 hatched: 13 reared to winged stage—(5 ♂ & 8 ♀).
 15th. 3.30 P.M. mating: continued to mate 15½ hours.
 16th. 15 eggs, 12 hatched: 6 reared to winged stage—(4 ♂ & 2 ♀).

AUGUST.

- 16th. 6.00 P.M. mating: continued to mate $9\frac{1}{2}$ hours.
 19th. 6.00 P.M. ditto ditto 15 hours.
 20th. 19 eggs, 15 hatched: 7 reared to winged stage—(6 ♂ & 1 ♀).
 22nd. 7.30 P.M. mating: continued to mate $13\frac{1}{2}$ hours.
 24th. 23 eggs, 15 hatched: 5 reared to winged stage—(3 ♂ & 2 ♀).
 24th. 5.45 P.M. mating: continued to mate $9\frac{3}{4}$ hours.
 25th. 2.00 P.M. ditto ditto $8\frac{1}{2}$ hours.
 26th. 5.30 P.M. ditto ditto $4\frac{1}{2}$ hours.
 27th. 5.30 P.M. ditto ditto 5 hours.
 28th. 7.30 P.M. ditto ditto $6\frac{3}{4}$ hours.
 30th. 8 eggs: 6 hatched (2 survived 2nd moult). These were later caged with nymphs from eggs deposited September 3rd and September 11th.
 30th. 4.30 P.M. mating: continued to mate $4\frac{1}{2}$ hours.
 31st. 3.45 P.M. ditto ditto $5\frac{1}{4}$ hours.

SEPTEMBER

- 1st. 6.15 A.M. ditto ditto $2\frac{1}{4}$ hours.
 1st. 9.00 P.M. ditto ditto $2\frac{1}{2}$ hours.
 2nd. 4.45 P.M. ditto ditto $7\frac{1}{4}$ hours.
 3rd. 7 eggs (4 survived 1st moult). These were later caged with nymphs from eggs deposited August 30th and September 11th.
 3rd. Noon, mating: continued to mate $3\frac{1}{2}$ hours.
 3rd. 4.30 P.M. mating: continued to mate $5\frac{1}{2}$ hours.
 4th. 3.00 P.M. ditto ditto 9 hours.
 5th. 6.15 P.M. ditto ditto $2\frac{1}{4}$ hours.
 8th. 7.30 P.M. ditto ditto $4\frac{1}{2}$ hours.
 9th. 5.15 P.M. ditto ditto $1\frac{1}{4}$ hours.
 11th. 15 eggs, 12 hatched (8 survived 2nd moult): added these to nymphs from eggs deposited Aug. 30th & Sept. 3rd. 10 reared to winged stage—(4 ♂ & 6 ♀).
 12th. 6.00 P.M. mating: continued to mate 11 hours.
 16th. The ♂ died: preserved in glycerine (tube 44). This male was photographed, and is shown on Plate 28, photo 14.
 21st. Killed the ♀. Preserved in glycerine (tube 44).

RECORD VI. CAGE 40.—1912.

Third pair of F_1 Hybrids.

JULY

- 24th. 5.00 A.M. mating: continued to mate $6\frac{1}{2}$ hours.
 30th. 28 eggs, all hatched: 15 reared to the winged stage—(5 ♂ & 10 ♀).
 31st. 5.00 A.M. mating: continued to mate 16 hours.

AUGUST

- 1st. 28 eggs: 25 survived 1st moult; 18 reared to winged stage—(11 ♂ & 7 ♀).
 3rd. 28 eggs: 20 survived 1st moult; 12 reared to winged stage—(6 ♂ & 6 ♀).
 6th. 6.00 P.M. mating: continued to mate 17 hours.
 11th. 7.30 P.M. ditto ditto 2 hours.
 16th. 5.00 A.M. ditto ditto 2 hours.

AUGUST

- 16th. 10.00 A.M. 28 eggs, all hatched : 17 reared to winged stage—(10 ♂ & 7 ♀).
 16th. 9.00 P.M. mating : continued to mate $6\frac{1}{2}$ hours.
 17th. 3.45 P.M. ditto ditto $5\frac{3}{4}$ hours.
 18th. 2.00 P.M. ditto ditto $7\frac{1}{2}$ hours.
 19th. 6.00 P.M. ditto ditto $3\frac{1}{2}$ hours.
 20th. 14 eggs. Killed 7 for cytological study ; the remaining 7 hatched. Added 6 that survived the 2nd moult to nymphs from eggs deposited August 27th. Reared the 6 to winged stage.
 20th. 7.30 P.M. mating : continued to mate 3 hours.
 21st. 1.30 P.M. ditto ditto 8 hours.
 22nd. 3.30 P.M. ditto ditto 8 hours.
 24th. 5.45 P.M. ditto ditto $4\frac{3}{4}$ hours.
 25th. 6.30 P.M. ditto ditto 4 hours.
 26th. 17 eggs, 16 hatched : 6 reared to winged stage—(4 ♂ & 2 ♀).
 26th. 5.30 P.M. mating : continued to mate 4 hours.
 27th. 10 eggs, all hatched : 9 reared to winged stage ; 15 in this cage including the 6 added from eggs deposited August 20th—(8 ♂ & 7 ♀).
 27th. 3.30 P.M. mating : continued to mate $6\frac{1}{2}$ hours.
 28th. 7.30 P.M. ditto ditto $5\frac{3}{4}$ hours.
 29th. 5.15 P.M. ditto ditto $4\frac{1}{4}$ hours.
 30th. 13 eggs, 12 hatched : 8 that survived were later caged with nymphs from eggs deposited September 7th.
 30th. 4.00 P.M. mating : continued to mate 7 hours.

SEPTEMBER

- 2nd. 15 eggs, all hatched : 10 reared to winged stage—(6 ♂ & 4 ♀).
 2nd. 4.45 P.M. mating : continued to mate $3\frac{3}{4}$ hours.
 3rd. 8.00 P.M. ditto ditto 1 hour.
 4th. The male died, and was preserved in glycerine (tube 38). This male is shown on Plate 28, photo 10.
 7th. 12 eggs, 11 hatched, and after the 2nd moult 8 nymphs were added from the eggs deposited August 30th. 17 reared to winged stage—(4 ♂ & 13 ♀).
 7th. Killed the female and preserved in glycerine (tube 38). We were forced to kill this female, as at this period we had as many nymphs as we could properly care for.

RECORD VII. CAGE 41.—1912.

Fourth Pair of F₁ Hybrids.

JULY

- 28th. 6.00 P.M. mating : continued to mate $12\frac{1}{4}$ hours.

AUGUST

- 8th. 20 eggs, 15 hatched : 8 reared to winged stage—(4 ♂ & 4 ♀).
 13th. 5.00 A.M. mating : continued to mate 31 hours.
 17th. 25 eggs, 22 hatched : 13 reared to winged stage—(7 ♂ and 6 ♀).
 22nd. 18 eggs, 15 hatched : 5 reared to winged stage—(1 ♂ & 4 ♀).
 23rd. 3.30 P.M. mating : continued to mate 40 hours.
 25th. 6.30 P.M. ditto ditto 17 hours.
 27th. 26 eggs, 21 hatched : 9 reared to winged stage—(2 ♂ & 7 ♀).
 27th. 5.30 P.M. mating : continued to mate $13\frac{1}{2}$ hours.

AUGUST

- 28th. 16 eggs, 13 survived 1st moult: These were later caged with nymphs from eggs deposited September 8th.
- 31st. 27 eggs, 23 survived 1st moult: 12 reared to winged stage—(4 ♂ & 8 ♀).
- 31st. 5.30 P.M. mating: continued to mate 11½ hours.

SEPTEMBER

- 5th. 24 eggs, 16 survived 1st moult: 7 reared to winged stage—(4 ♂ & 3 ♀).
- 5th. 6.15 P.M. mating: continued to mate 49 hours.
- 8th. 14 eggs (7 killed for cytological study): 5 hatched; added these to nymphs from eggs deposited August 28th. (18 in all.) 9 reared to winged stage—(6 ♂ & 3 ♀).
- 8th. 2.30 P.M. mating: continued to mate 11½ hours.
- 9th. 5.15 P.M. ditto : ditto 6 hours.
- 10th. 7.00 P.M. ditto : ditto 2 hours.
- 11th. Killed both ♂ and ♀, and preserved in glycerine (tube 42). The male is shown on Plate 28, photo 12. We were forced to kill this pair, as we had as many nymphs in the laboratory as we could properly care for.

RECORD VIII. CAGE 44.—1912.

Fifth Pair of F₁ Hybrids.

AUGUST

- 6th. 2.00 P.M. mating: continued to mate 39 hours.
- 8th. 25 eggs, 2 hatched: none reared to winged stage.
- 9th. 5.00 P.M. mating: continued to mate 4 hours.
- 11th. 5.00 A.M. ditto ditto 1 hour.
- 14th. 13 eggs (1 group of 10 and 1 group of 3): 9 hatched, 7 survived 3rd moult. These were caged later with nymphs from eggs deposited August 20th.
- 15th. 6.00 P.M. mating: continued to mate 14 hours.
- 19th. 5.00 A.M. ditto ditto 4½ hours.
- 20th. 13 eggs: 8 survived 1st moult, 4 survived 2nd moult. Added to nymphs from eggs deposited August 14th. 8 reared to winged stage—(1 ♂ & 7 ♀).
- 23rd. 3.30 P.M. mating: continued to mate 16 hours.
- 27th. 30 eggs: one hatched. Did not rear.
- 27th. 7.30 P.M. mating: continued to mate 14½ hours.
- 28th. 7.30 P.M. ditto ditto 9¾ hours.
- 29th. 15 eggs: 11 survived 1st moult, 4 survived 3rd moult. These were later caged with nymphs from eggs deposited September 3rd.

SEPTEMBER

- 1st. 9.00 P.M. mating: continued to mate 7 hours.
- 2nd. 4.45 P.M. ditto ditto 7¼ hours.
- 3rd. 14 eggs, 8 hatched: 5 survived 2nd moult, added these to nymphs from eggs deposited August 29th; 8 reared to winged stage—(3 ♂ & 5 ♀).
- 3rd. Killed both the ♂ & ♀, and preserved them in glycerine (tube 37). The male is shown on Plate 28, photo 11.

We killed this pair because the small percentage of eggs that had hatched indicated that these bugs were not functioning normally.

RECORD IX. CAGE 46.—1912.

Sixth Pair of F₁ Hybrids.

(This ♀ and the ♀ of Cage 48 were fertilized by the same ♂.)

AUGUST

- 15th. 3.30 P.M. mating: continued to mate 15½ hours.
 22nd. 21 eggs, 6 hatched (only 2 survived the 1st moult): later added these to nymphs from eggs deposited August 27th.
 27th. 9 eggs, all hatched: added these to nymphs from eggs deposited August 22nd. 5 reared to winged stage—(2 ♂ & 3 ♀).
 27th. 7.30 P.M. mating: continued to mate 3 hours.
 29th. 14 eggs, 1 hatched and died after 1st moult.
 29th. 5.15 P.M. mating: continued to mate 4¼ hours.

SEPTEMBER

- 3rd. 14 eggs. All fertilized, but none hatched.
 3rd. 2.30 P.M. mating: continued to mate 7½ hours.
 4th. 12 eggs: 7 fertilized, but none hatched.
 4th. 3.00 P.M. mating: continued to mate 6 hours.
 5th. 6.15 P.M. ditto ditto 1¼ hours.
 8th. 19 eggs, 5 hatched. Later added these to nymphs from eggs deposited September 11th and September 14th.
 11th. 11 eggs, 10 hatched, 8 survived first moult. Added these to nymphs from eggs deposited September 8th and September 14th.
 11th. 5.45 P.M. mating: continued to mate 5¾ hours.
 12th. 6.00 P.M. ditto ditto 4 hours.
 13th. 6.00 P.M. ditto ditto 2½ hours.
 14th. 14 eggs, nearly all fertilized, 3 hatched. Added these to nymphs from eggs deposited September 8th and September 11th. 8 reared to winged stage—(5 ♂ & 3 ♀).
 17th. 12 eggs: 11 fertilized, but none hatched.
 17th. 12.45 P.M. mating: continued to mate 7¾ hours.
 18th. 8.00 P.M. ditto ditto 5½ hours.
 19th. 8 eggs: 4 fertilized, none hatched.
 19th. Killed the male and preserved in glycerine (tube 49). This male was photographed, and is shown on Plate 28, photo 13.
 21st. Killed the female and preserved in glycerine (tube 49).

RECORD X. CAGE 48.—1912.

Seventh Pair of F₁ Hybrids.

(This ♀ and the ♀ of Cage 46 were fertilized by the same ♂.)

AUGUST

- 16th. 9.00 P.M. mating: continued to mate 8 hours.
 19th. 5.00 A.M. ditto ditto 5 hours.
 20th. 21 eggs, 20 hatched: 15 reared to winged stage—(6 ♂ & 9 ♀).
 22nd. 15 eggs: 13 survived first moult. 10 reared to winged stage—(4 ♂ & 6 ♀).

AUGUST

- 26th. 5.00 A.M., 28 eggs (one group of 16 and one group of 12): 26 hatched, 14 reared to winged stage—(3 ♂ & 11 ♀).
- 27th. 5.30 A.M. mating: continued to mate 16½ hours.
- 28th. 14 eggs, 4 hatched. These were caged later with nymphs from eggs deposited August 31st.
- 31st. 14 eggs, 14 hatched. Added these to nymphs from eggs deposited August 28th. 12 reared to winged stage—(10 ♂ & 2 ♀).
- 31st. 3.30 P.M. mating: continued to mate 9¼ hours.

SEPTEMBER

- 1st. 9.00 P.M. ditto ditto 8 hours.
- 2nd. 14 eggs, 13 hatched. 9 reared to winged stage—(6 ♂ & 3 ♀).
- 2nd. 4.45 P.M. mating: continued to mate 3¼ hours.
- 5th. 10 eggs, all hatched. These were caged later with nymphs from eggs deposited September 8th.
- 7th. 4.30 P.M. mating: continued to mate 3 hours.
- 8th. 4 eggs, all hatched. Added these to nymphs from eggs deposited September 5th. 8 reared to winged stage—(5 ♂ & 3 ♀).
- 8th. 7.30 P.M. mating: continued to mate 2 hours.
- 17th. Killed the female, as she had not mated nor deposited eggs for 9 days: preserved in glycerine (tube 46). The male is shown on Plate 28, photo 13.

RECORD XI. CAGE 50.—1912.

One Pair F₁ Hybrid ♀ × Pure *variolaris* ♂.

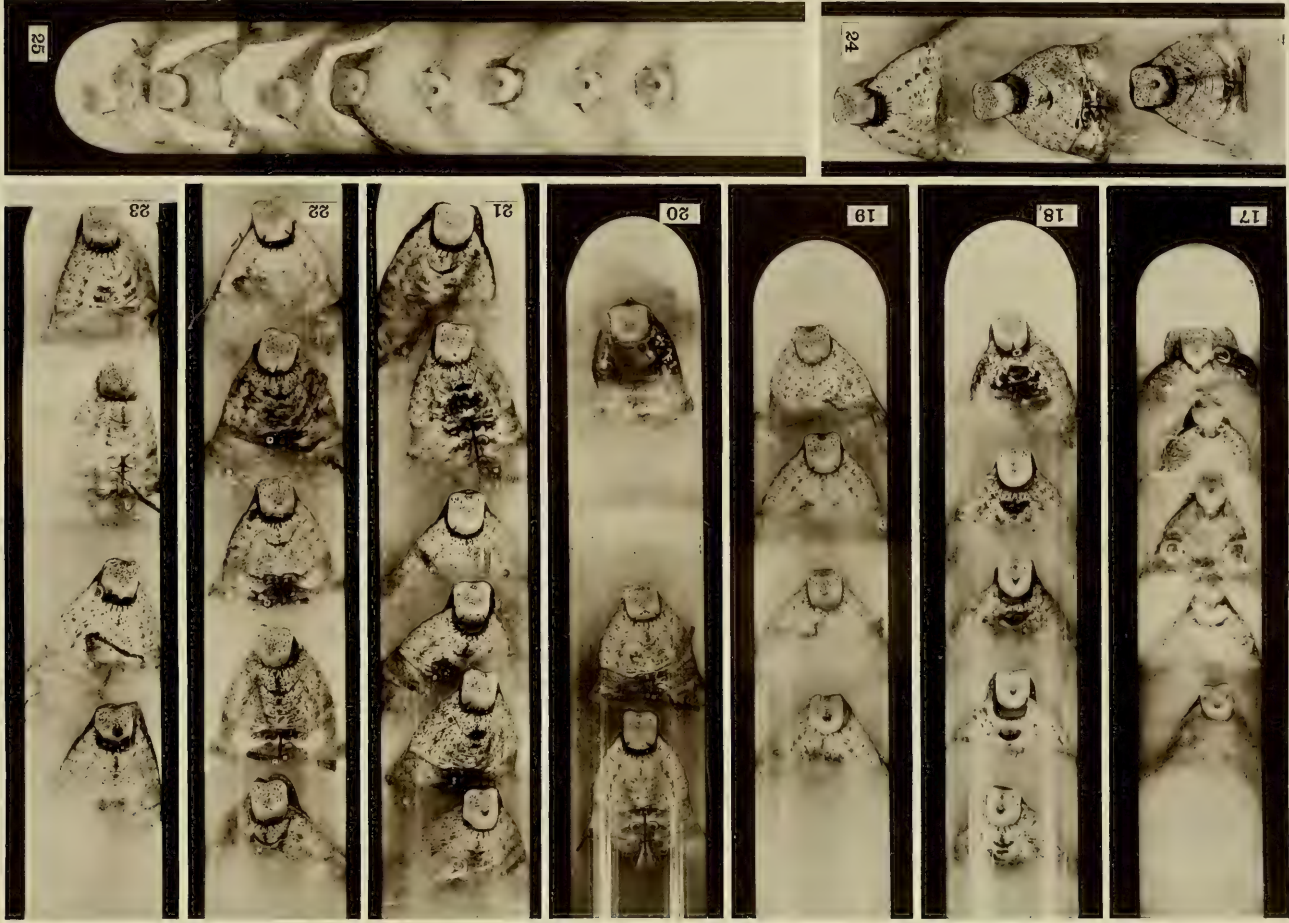
AUGUST

- 22nd. 7.30 p.m. mating: continued to mate 13 hours.
- 25th. 9 eggs: 7 developed, but failed to hatch.
- 27th. 11 eggs: all developed, 2 hatched. After second moult, these were caged with nymphs from eggs deposited September 2nd and September 3rd.
- 28th. 15 eggs: 13 developed, but none hatched.
- 30th. 14 eggs, 10 hatched. 9 reared to winged stage—(7 ♂ & 2 ♀).

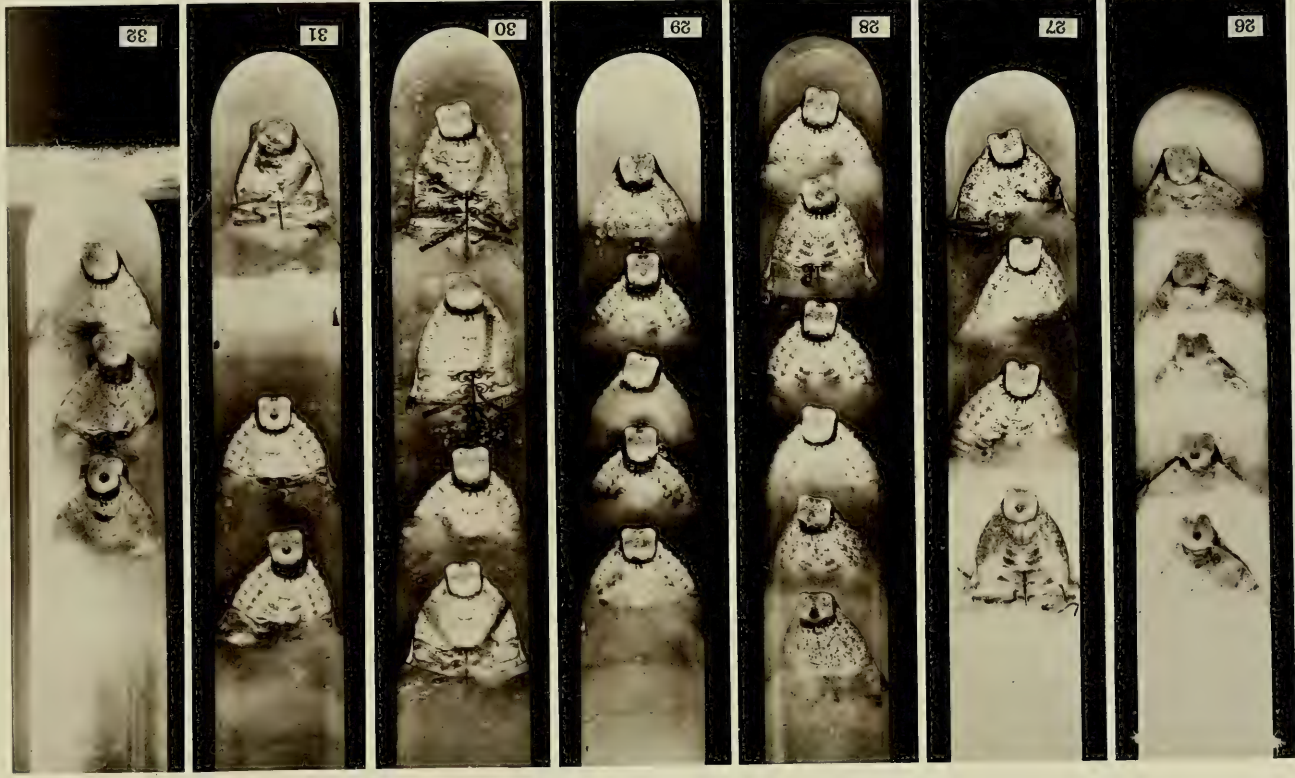
SEPTEMBER

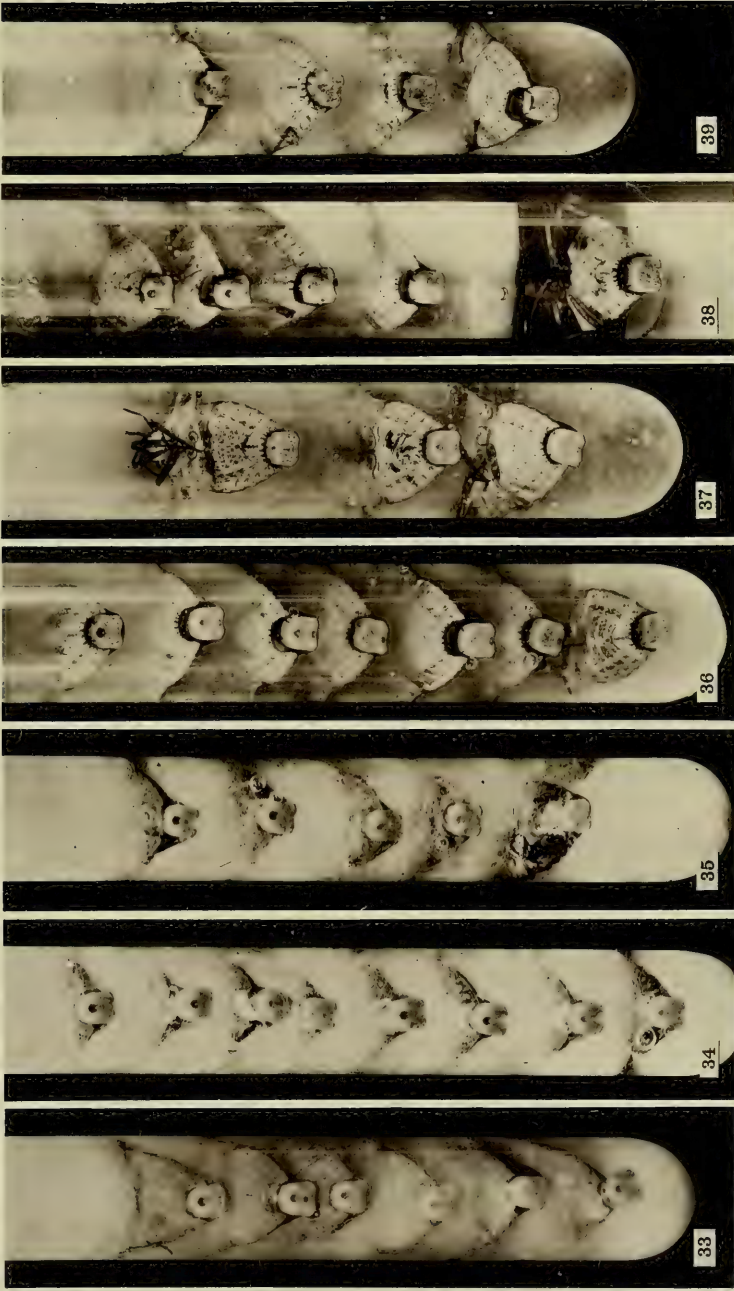
- 2nd. 6 eggs, 5 developed, but only 1 hatched. After first moult, this was caged with nymphs from eggs deposited August 27th and September 3rd.
- 3rd. 8 eggs, all developed, 3 hatched. Added these, after first moult, to nymphs from eggs deposited August 27th and September 2nd. 4 reared to winged stage—(3 ♂ & 1 ♀).
- 4th. 13 eggs, 5 hatched. These were caged later with nymphs from eggs deposited September 16th and September 18th.
- 8th. 11 eggs, all hatched: 7 reared to winged stage—(3 ♂ & 4 ♀).
- 11th. 14 eggs, 13 developed, but none hatched.
- 13th. 4 eggs, all developed, but none hatched.
- 16th. 10 eggs, 9 developed, 2 hatched. Added these later to nymphs from eggs deposited September 4th and 18th.
- 18th. 4 eggs, 2 hatched. Added these, after second moult, to nymphs from eggs deposited September 4th and September 16th. 6 reared to winged stage—(5 ♂ & 1 ♀).
- Both parents killed and preserved in glycerine (tube 47). The male was photographed, and is shown on Plate 34, photo 58.





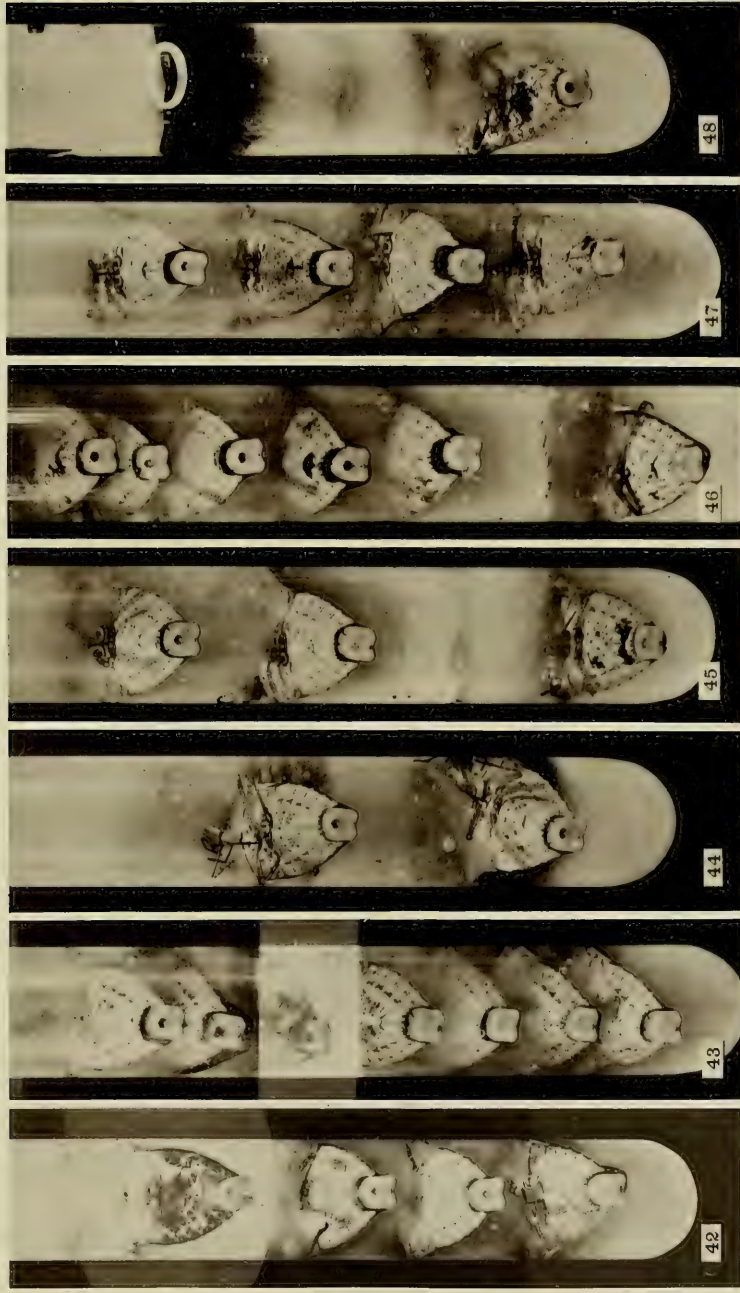
F₂ HYBRIDS from *E. VARIOLARIUS* & *E. SERVUS*.





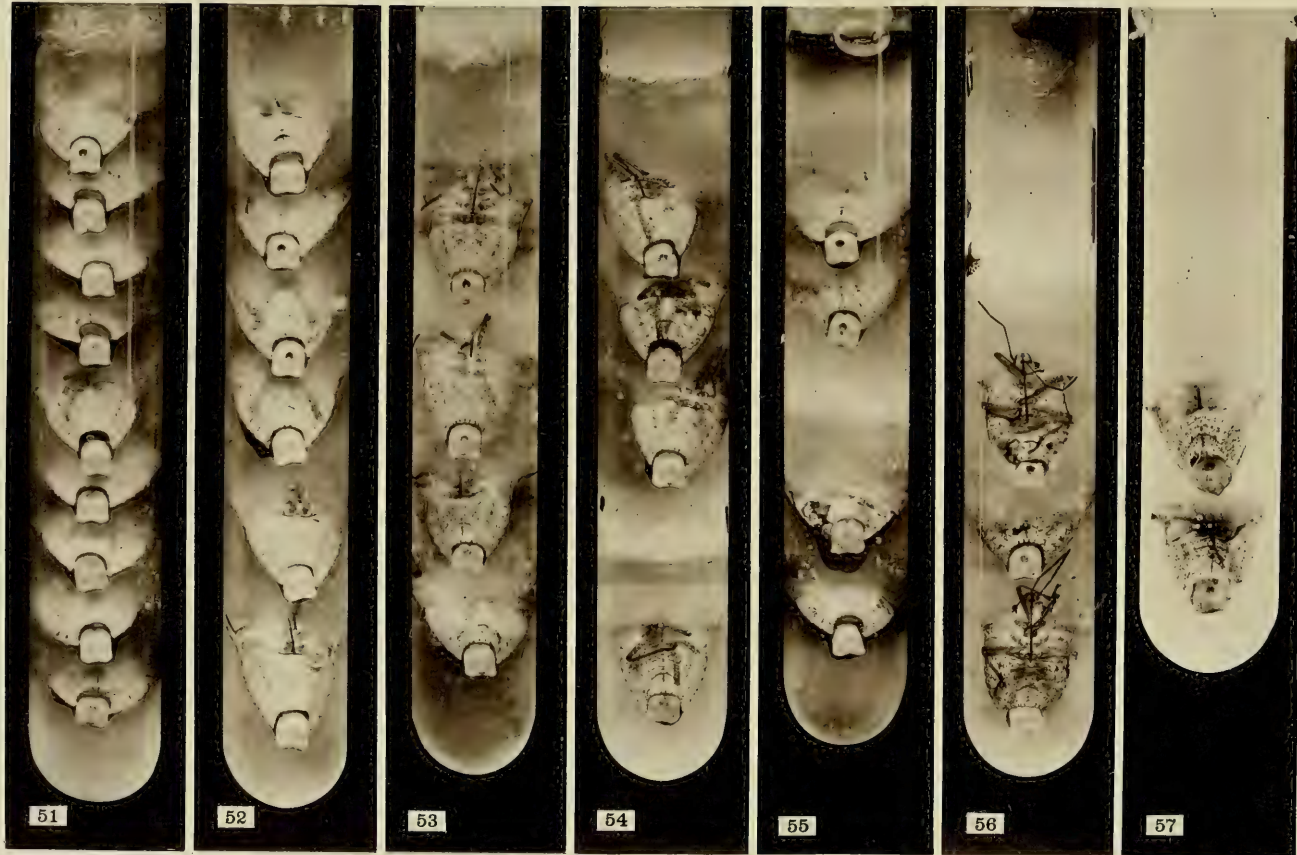
F₂ HYBRIDS from E. VARIOLARIUS & E. SERVUS.

Anglo Engraving Co.



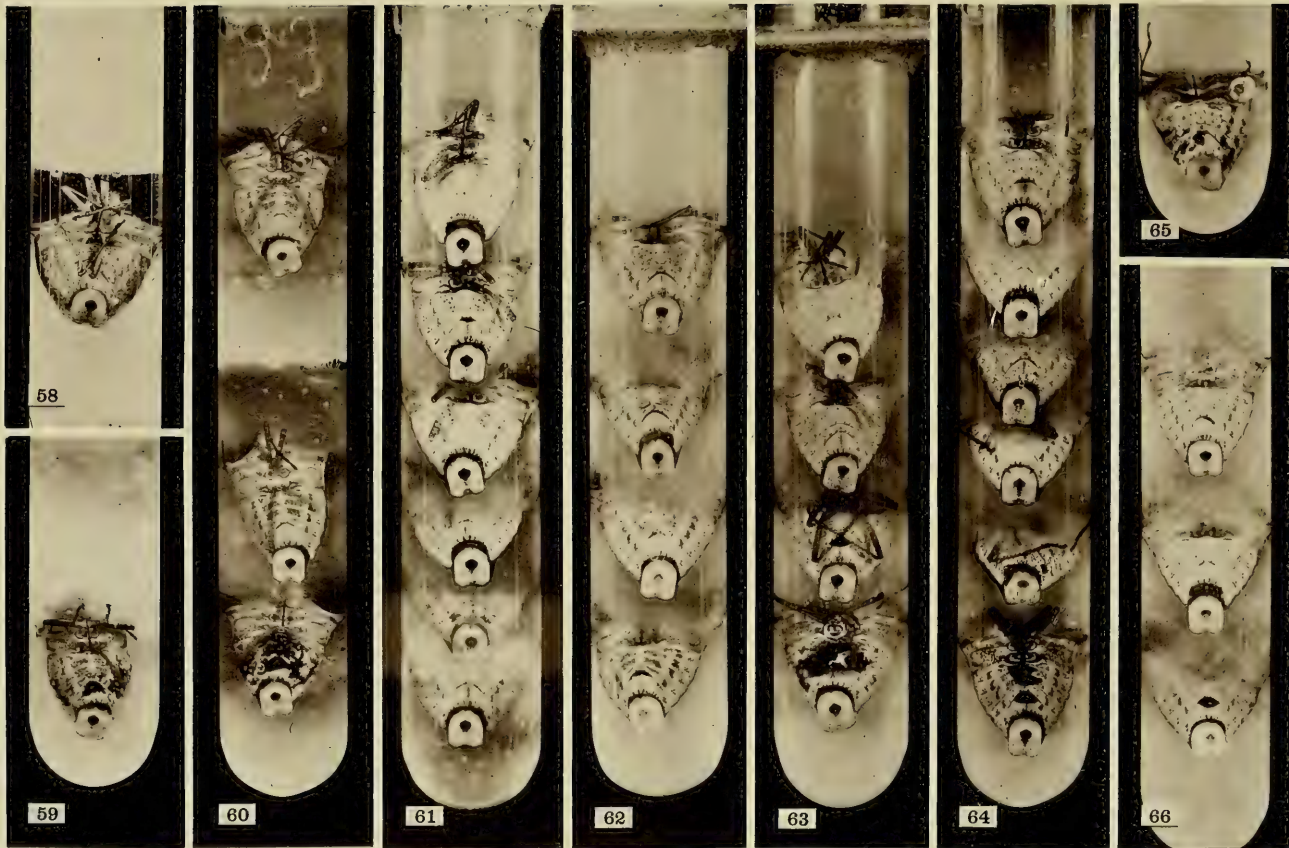
F₂ HYBRIDS from E. VARIOLARIUS & E. SERVUS.

Anglo Engraving Co.



Anglo Engraving Co.

F₂ HYBRIDS from *E. VARIOLARIUS* & *E. SERVUS*.



Anglo Engraving Co.

E. VARIOLARIUS MALES, & MALES from F1 ♀ X E. VARIOLARIUS ♂

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EXPLANATION OF THE PLATES.

All the photographs are of male specimens. In all cases only the ventral surface of the bugs is shown.

The two bugs of photo 1 are dried specimens. Those of photos 2 to 66 are preserved in glycerine*. The specimens are placed serially in a small test-tube which is filled with pure glycerine and hermetically sealed. The genital segment of each bug has been pulled out and cotton inserted behind the segment to hold it in position to show the entire ventral surface.

The magnification is about $1\frac{1}{2}$ diameters: this varies slightly because the work was done by two photographers, and they did not give exactly the same enlargement. The reproductions are bromide prints made directly from large negatives of the original plates; the individual photographs of which these plates were composed being sunprints from the original negatives.

PLATE 23.

- PHOTO 1. Male specimens of *Euschistus variolarius* and *Euschistus servus*. On the left is *E. variolarius*, showing the ventral surface and the clearly defined black spot, always present on the genital segment of the males of this species. On the right is *E. servus*, showing the ventral surface and the genital segment without any trace of the black spot typical of *variolarius*.
- PHOTO 2. Seven male specimens of *E. variolarius*, showing the typical black spot on the genital segment. These specimens are from the first generation of 1912. The parent bugs were of the first generation of 1911, which were kept in captivity through the winter of 1911-12.
- PHOTO 3. Five male specimens of *E. servus* received from North Carolina in the fall of 1912.
- PHOTO 4. The wild *E. servus* male that fertilized the *E. variolarius* female.
- PHOTO 5. Three of the five *E. servus* males that were caged all winter with *E. variolarius* females: see page 342.

* We are indebted to Prof. Ralph Tower, of the Museum of Natural History of New York, for suggesting the use of glycerine as a preservative.

- PHOTO 6. One of the two male *E. variolarius* from the same deposition of eggs from which we raised the three females for crossing with *E. servus*: see page 342.
- PHOTO 7. Two male specimens of the F_1 hybrid generation. These bugs were killed August 11th, 1912, and their testes dissected out and mounted for cytological study.
- PHOTO 8. Two male specimens of the F_1 hybrid generation. Killed August 28th, 1912.
- PHOTO 9. The male of the first pair of F_1 hybrids. The F_2 males from this pair of hybrids are shown on Plate 29, photos 17-25. See Record IV. and page 346.
- PHOTO 10. The male of the third pair of F_1 hybrids. The F_2 males from this pair of hybrids are shown on Plate 31, photos 33-41. See Record VI. and page 347.
- PHOTO 11. The male of the fifth pair of F_1 hybrids. The F_2 males from this pair of hybrids are shown on Plate 28, photos 15 and 16. See Record VIII. and page 347.
- PHOTO 12. The male of the fourth pair of F_1 hybrids. The F_2 males from this pair of hybrids are shown on Plate 32, photos 42-48. See Record VII. and page 347.
- PHOTO 13. The male of the sixth and seventh pairs of F_1 hybrids. The F_2 males from the sixth pair are shown on Plate 32, photos 49 & 50, and the F_2 males from the seventh pair are shown on Plate 33, photos 51-57. See Records IX. and X. and page 349.
- PHOTO 14. The male of the second pair of F_1 hybrids. The F_2 males from this pair are shown on Plate 30, photos 26-32. See Record V. and page 346.
- PHOTOS 15-16. Four F_2 males from the fifth pair of F_1 hybrids. See photo 11 for the male of this pair of hybrids, Record VIII. and page 347.

PLATE 29.

- PHOTOS 17-25. Forty-three F_2 males from the first pair of F_1 hybrids. See photo 9, Plate 28, for the male of this pair of hybrids, Record IV. and page 346.

PLATE 30.

- PHOTOS 26-32. Thirty F_2 males from the second pair of F_1 hybrids. See photo 14, Plate 28, for the male of this pair of F_1 hybrids, Record V. and page 346.

PLATE 31.

- PHOTOS 33-41. Forty-eight F_2 males from the third pair of F_1 hybrids. See photo 10, Plate 28, for the male of this pair of hybrids, Record VI. and page 347.

PLATE 32.

- PHOTOS 42-48. Twenty-seven F_2 males from the fourth pair of F_1 hybrids. See photo 12, Plate 28, for the male of this pair of hybrids, Record VII. and page 347.

- PHOTOS 49-50. Six F_2 males from the sixth pair of F_1 hybrids. See photo 13, Plate 28, for the male of this pair of hybrids, Record IX. and page 349.

PLATE 33.

- PHOTOS 51-57. Thirty-two F_2 males from the seventh pair of F_1 hybrids. See photo 13, Plate 28, for the male of this pair of hybrids, Record X. and page 349.

PLATE 34.

- PHOTO 58. The pure male *variolarius* that was raised in the laboratory in 1912, and the same season fertilized both a pure female *variolarius* and the F₁ hybrid female of Record XI, and pages 345, 353.
- PHOTOS 59-61. Ten males from the above mentioned pair of pure *variolarius*. See photo 58 for the male of this pair.
- PHOTOS 62-66. Eighteen males from the above mentioned F₁ hybrid female, fertilized by the pure *variolarius* male of photo 58, Record XI. and pages 345, 352-3.